CHARACTERZING THE EPICENTRAL TENDON-VERTEBRA FIBROUS ENTHESIS IN RAINBOW TROUT

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Introduction

- Rainbow trout body undulation plays an essential role in swimming mechanics
- It is achieved through the epicentral tendon (ECT) transferring force between contracting red and white muscles to the vertebrate backbone (Fig 1B)
- The ECT attaches to bone through a fibrous enthesis, which is an insertion site consisting of dense fibrous CT and lacks fibrocartilage (Benjamin et al., 2002)
- Soft-hard tissue interfaces with large mismatches in material properties like tendon and bone are known to be subject to increased chances of failure due to increased stress concentrations (Pilkey & Pilkey, 2020)
- Despite this, fibrous entheses both in mammals and fish show unexpected durability, and bone avulsions or tendon ruptures tend to occur before the enthesis fails (McCoy & Nelson, 2020; MacMaster, 2020)

Research question: How do the structural features of the fibrous entheses reduce stress concentrations between soft and hard tissue interfaces like tendon and bone?

- Previous work from our laboratory has revealed an uncharacterized cell type with large, rounded nuclei (termed *cell of interest*; COI) localized at the ECT enthesis (Fig 2D) (MacMaster, 2020)
- This cell could potentially contribute to reduced stress concentrations occurring between tendon-bone interfaces within the ECT fibrous enthesis

Objectives

- This is a descriptive observational study using histological assessments to characterize the ECT enthesis at the microscopic level with focus on the uncharacterized cell type and tendon extracellular matrix

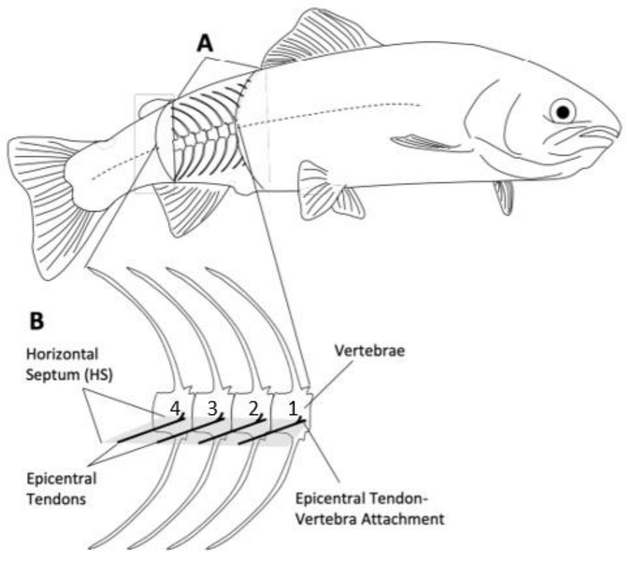

Figure 1: The arrangement of the ECTs within the horizontal septum of rainbow trout and their attachment to vertebra. A, schematic illustrating the region of interest from which all epicentral tendon-vertebrae attachments were obtained for histomorphometric analysis. We refer to this region as the *dorsal-adipose tissue segment*. B, a lateral view of epicentral tendon-vertebrae attachments on one side of the fish numbered in the rostral-caudal direction. ECT connection to red or white muscle not shown. This figure was taken directly from Emily Macmaster's thesis (MacMaster, 2020). BV = Bone Vertebra, T = Tendon.

Methods

Tissue section preparation

- Ten epicentral tendons taken from the *dorsal-adipose tissue segment* (Fig 1A) of 3 different fish were sectioned in the rostral-caudal direction to give 5 µm thick serial slide sections
- Tissue sections were then stained with H&E and pictures of the enthesis were taken through a compound microscope to be analyzed using ImageJ for quantitative and qualitative measurements

Quantitative measures

- Various regions of the ECT enthesis were defined along 3 different axes (anterior-posterior, dorsal-ventral and medial-lateral axes) for purposes of histomorphometric analysis (Fig 2-3)

COI density analysis

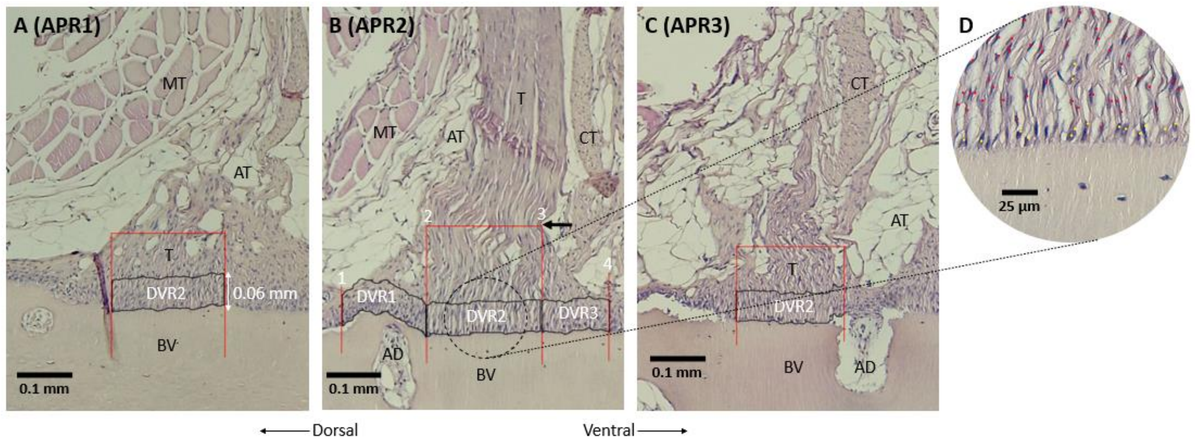

Figure 2: Defined regions for COI density analysis along the tendon side of the ECT enthesis. A, DVR2 defined within APR1 of the tendon attachment. B, 3 DVRs defined along the dorsal to ventral axis of the tendon attachment within APR2. The black arrow indicates the point where tendon fibers begin to splay outward. C, DVR2 defined within APR3 of the tendon attachment. The different DVRs are outlines in black and red lines illustrate how each DVR placement was determined. D, zoomed in view of DVR2 within APR2 of the tendon attachment highlighting the difference between nuclei of COIs (yellow) and nuclei of other cells (red). Only yellow highlighted cells were counted. Photos were taken at 10X (A, B, C) and 40X (D) magnification. BV = Bone Vertebra, T = Tendon, AT = Adipocyte Tissue, AD = Adipocyte Deposit and CT = Connective Tissue, MT = Muscle Tissue.

Tendon fiber compaction analysis

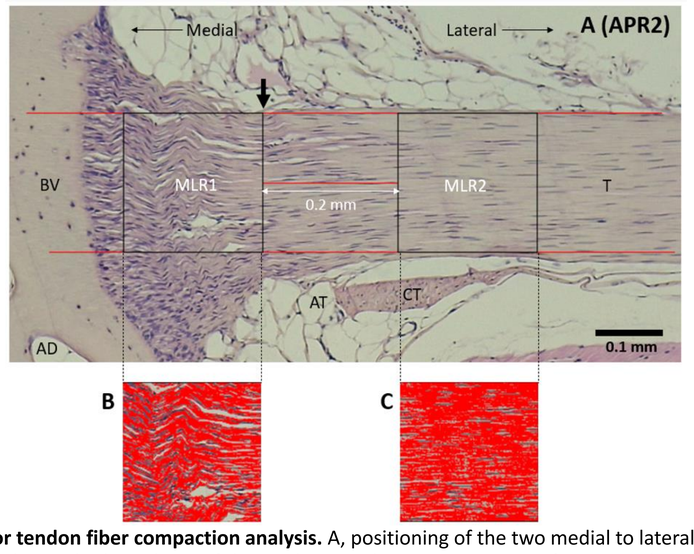

Figure 3: Defined regions for tendon fiber compaction analysis. A, positioning of the two medial to lateral regions (termed ML regions; MLR 1 and 2) along the medio-lateral axis of the tendon attachment at 10X magnification within APR2. The different MLRs are outlines in black and red lines illustrate how each MLR placement was determined. The black arrow indicates the point where tendon fibers begin to splay outward. B, C, MLR1 and MLR2 highlighted with the color threshold tool on ImageJ at 10X magnification. BV = Bone Vertebra, T = Tendon, AT = Adipocyte Tissue, AD = Adipocyte Deposit and CT = Connective Tissue.

Qualitative observations

- Additionally, various qualitative observations were noted including morphology and arrangement of COI nuclei, tendon fiber orientation, tendon-bone interface convolution, adipocyte deposits/tissue and tendon branching

Statistical analysis

- Statistical analysis was completed using R v4.0.2
- For COI cell density analysis, 2 separate parametric one-way ANOVAs were conducted treating each factor as a fixed effect with post hoc multiple comparisons adjusted for using Bonferroni
- For tendon fiber compaction analysis, a parametric paired t-test was conducted
- P-values and family-wise p-values < 0.05 were considered statistically significant

Results

COI density

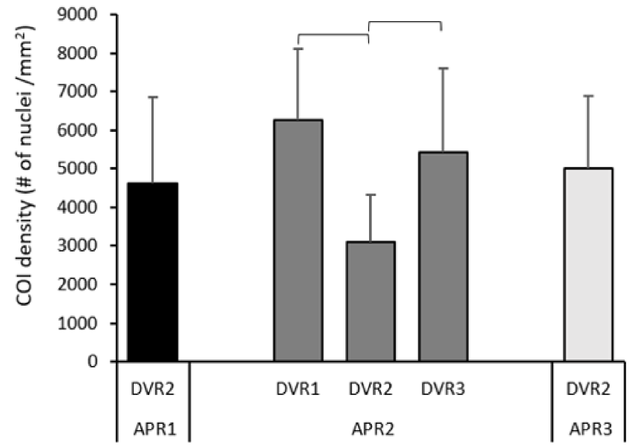

Figure 4: Results for COI density analysis along the anterior to posterior and dorsal to ventral axes of the ECT enthesis. Quantification of COI density was done in DVR2 for each APR and within APR2 for each DVR by performing manual COI counts and dividing the number of COIs by the area occupied by the region. Brackets denote significant differences ($p < 0.05$, Bonferroni post hoc) in mean COI density between the different DVRs within APR2. Bar graphs represent the COI density means + SD, $\alpha = 0.05$. Abbreviations: DVR = dorsal-ventral region, APR = anterior-posterior region.

Tendon fiber compaction

Table 1: Mean estimated tendon fiber percentage within the MLR's of the ECT enthesis. The tendon fiber percentage is displayed as the mean ± SD (n = 10 for both the ML1 and ML2 regions). Pairwise t-test gave a p-value of 0.0009. Abbreviations: MLR, medial-lateral region.

MLR	Tendon Fiber Percentage
MLR1	53.06 ± 4.91
MLR2	68.07 ± 12.94

Conclusions

- COI density is greater in the splaying edges (DVR1 and 3) than enthesis center (DVR2) (Fig 4)
- Tendon fiber compaction is lower proximal to the enthesis interface (MLR1) compared to more distal tendon tissue (MLR2) (Table 1)
- Histomorphometric analysis reveal details of the enthesis morphology with the potential to reduce stress concentrations at the tendon-bone interface

Future directions

- Additional histological assessments are needed to further characterize the unknown cell type and surrounding extracellular matrix composition using immunohistochemistry and laser microdissection

References

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