Matrix Microarchitecture Drives Cellular Function and Extracellular Vesicle Proteome in Engineered Tendon Models

Kariman Shama[1], Zachary Greenberg[2], Mei He[1,2], Brittany Taylor[1,3]
[1]J. Crayton Pruitt Family Department of Biomedical Engineering, Gainesville, FL, [2]Department of Pharmaceutics, Gainesville, FL, [3]Department of Pharmacology and Therapeutics, Gainesville, FL
k.shama@ufl.edu

Disclosures: Kariman Shama (N), Zachary Greenberg (N), Mei He (N), Brittany Taylor (N)

INTRODUCTION: Tendinopathy is a common musculoskeletal disorder marked by pain, impaired function, and extracellular matrix (ECM) disorganization. At the cellular level, tendon-derived cells become hypercellular and lose their native spindle-like morphology, reflecting disease progression. Emerging evidence suggests that extracellular vesicles (EVs), cell-secreted nanoparticles that mediate intercellular communication, play critical roles in musculoskeletal pathology by regulating inflammation, ECM remodeling, and cellular behavior [1]. While EVs have recognized regenerative roles, their contributions to tendon pathology remain unclear. In vitro tendon models provide controlled systems to capture early disease processes that are difficult to study in vivo. Our objective is to define how matrix microarchitecture influences cellular behavior and EV cargo in tendon disease, with the hypothesis that diseased-mimetic environments drive distinct EV signatures linked to fibrosis and inflammation, establishing EVs as biomarkers and therapeutic targets. Previously, we have demonstrated that EV profiles change due to nanofibrous microarchitecture. Here, we employ biomimetic in vitro models of healthy and diseased tendon to investigate temporal changes in EV cargo and validate tendon cell phenotypes. We hypothesized that diseased mimetic cultures would show increased proliferation, elevated matrix remodeling markers, and EVs with greater secretion, remodeling protein enrichment, and distinct proteomic profiles.

METHODS: Electrospun polycaprolactone (PCL) scaffolds were fabricated to mimic healthy (aligned) and diseased (disorganized) tendon microenvironments. Primary tendon-derived cells (TDCs) were isolated from 9-month-old male and female Sprague-Dawley rat Achilles tendons, expanded, and seeded onto scaffolds (~52,000 cells/cm^2). Cell proliferation, morphology, and tendon remodeling markers (collagen I, collagen III, fibronectin) were assessed via DNA/MTS assays, immunofluorescence, and western blot. Extracellular vesicles (EVs) were isolated from conditioned media using a membrane affinity-based kit, characterized by nanoparticle tracking analysis and transmission electron microscopy, and subjected to proteomic profiling by LC–MS/MS. Differential protein expression and pathway enrichment analyses were performed to identify EV-mediated signaling changes associated with tendon pathology. Statistical analyses were performed using GraphPad Prism applying ANOVA (one- or two-way with Tukey's post hoc), ANCOVA, and normality tests to evaluate cellular, EV, and proteomic data, with differentially expressed proteins defined by FDR < 0.05.

RESULTS SECTION: Primary tendon-derived cells on healthy scaffolds exhibited greater elongation and alignment compared to monolayers, while diseased scaffolds promoted enhanced proliferation and progressive alignment over time **(Figure 1)**. Diseased models showed elevated expression of fibronectin and collagen III, consistent with tendon pathology, whereas collagen I peaked earlier in healthy scaffolds. EV analyses revealed distinct remodeling-associated signatures: diseased EVs were enriched in collagen III and temporally upregulated proteins linked to ECM remodeling, cytoskeletal dynamics, and inflammation (e.g., ANXA1, LGALS1, MMP2, INHBA) **(Figure 2).** These findings demonstrate that diseased microenvironments drive both cellular and EV-mediated remodeling processes reflective of tendinopathy.

DISCUSSION: Tendon-derived cells exhibited microenvironment-dependent behaviors where healthy, aligned scaffolds promoted spindle-like elongation and alignment consistent with native tendon architecture, whereas diseased, disorganized scaffolds drove hypercellularity, late-stage alignment, and features of fibrotic remodeling. These patterns mirror histopathology in tendinopathy, including collagen disorganization and neovascularization [2,3]. Temporal ECM shifts tracked disease state where fibronectin and collagen III were upregulated persistently in diseased conditions, while collagen I was upregulated at earlier timepoints; aligning with studies linking fibronectin/Col I accumulation to aberrant matrix remodeling and impaired mechanics in chronic tendon injury [4]. EV analyses revealed complementary, disease-skewed signatures. Cytoskeletal proteins such as vimentin and annexin A2, ECM/adhesion mediators including versican and nidogen-1, and immunomodulatory/remodeling proteins such as annexin A1 and MMP2 were enriched. These findings are consistent with EVs acting as conveyors of matrix and inflammatory cues that potentiate tissue remodeling [5–8]. Late-stage enrichment of INHBA (activin A subunit) and metabolic stress markers such as ENO1 supports a progression toward profibrotic signaling and glycolytic reprogramming, processes increasingly implicated in fibrotic disease across tissues [9–12]. Collectively, these data position EV cargo as a sensitive readout of tendon microenvironmental state and a practical avenue for biomarker discovery, while highlighting EV-mediated pathways such as MMP-linked remodeling and activin/TGF-β–related signaling as rational therapeutic targets to interrupt or reverse fibrotic remodeling in tendinopathy [8–12].

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes a 3D tendon model that captures healthy and diseased microenvironments, revealing dynamic changes in cell behavior and EV cargo linked to fibrosis and inflammation. These insights support the use of EVs as biomarkers for early diagnosis and monitoring, while identifying EV-mediated pathways as therapeutic targets to prevent or reverse tendon fibrosis.

REFERENCES: [1] Eslami F. et al. Heliyon 2024 [2] Khan KM et al. BMJ. 2002 [3] JD Rees et al. Rheumatology 2006 [4] Kubow KE et al. Nat Commun. 2015 [5] F Danielsson et al. Cells 2018 [6] V Gerke, Physiol Rev. 2002 [7] G Leoni et al. J Clin Invest. 2015 [8] E. Huisman et al. J Orthop Res. 2016 [9] M Wu et al. BMC Complement Med. Ther. 2022 [10] Jiang K et al. Cell Death & Disease 2020 [11] Huang T. et al. World J Gastroenterol 2022 [12] Lee Y, Kim JH.Clin Mol Hepatol. 2022

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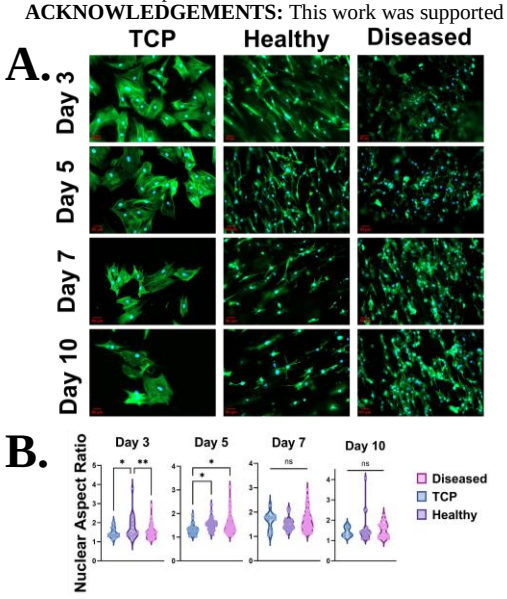

Figure 1: (A) Actin (green) and DAPI (blue) staining of tendon fibroblasts on TCP controls, healthy, and diseased mimetic models (n=3). (B) Nuclear aspect ratio was quantified in ImageJ by measuring nuclear major and minor axes. p < 0.05 (*) and p < 0.01 (**) and data reported as mean ± S.D.

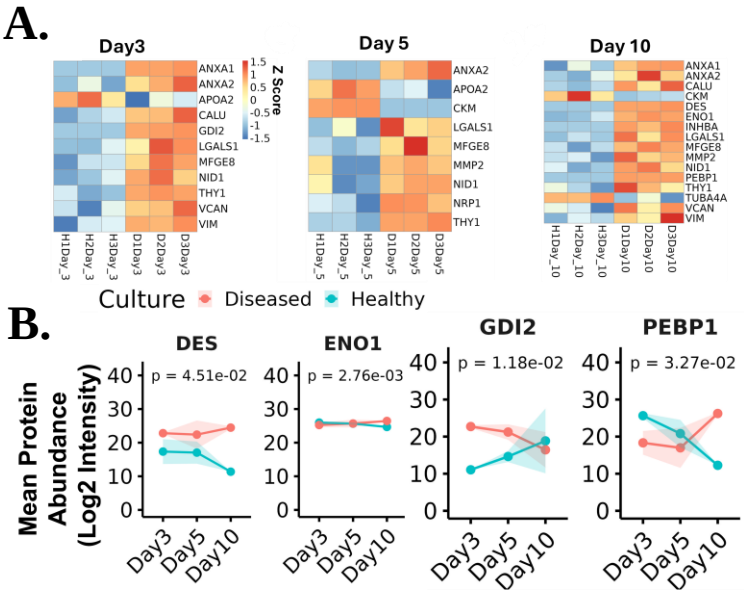

Figure 2: (A) Heatmaps depicting differentially enriched proteins at day 3, day 5, day 7, and day 10 respectively. (B) Time-course analysis of ENO1 and PEBP1 protein expression, highlighting their temporal regulation.