Tensile Strain Conditioning Affects the Vascular Morphology of 3D Microvascular Constructs

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Disclosures: None.

INTRODUCTION: Revascularization is a critical process for tissue regeneration following injury. Emerging research has focused on the therapeutic benefits of mechanical loading that promote angiogenesis, the process of forming new capillary networks from preexisting blood vessels. [1] Recent work demonstrated that the magnitude, initiation time, and modality of compressive strain impacts vascular network formation *in vitro*. [2] However, these conditioning behaviors have yet to be fully assessed in tension, which is the primary loading orientation for vascularized soft tissue. In addition, human gait involves cyclic loading at 0.6-1.4Hz with a duration that can vary. [3] Therefore, we developed a custom stainless-steel assembly that enables both the casting of cell-encapsulated hydrogels and the anchoring between two stainless steel meshes for displacement-controlled tensile strain conditioning with an 8 hour per day duration. In this work, we sought to modulate the type of tensile strain conditioning, either 10% static or 10% cyclic at 0.8Hz, and assess volumetric vascular morphology of adipose-derived microvascular fragments (MVFs) seeded in collagen type-I. We hypothesized that 10% static tensile strain would enhance MVF network length and branching at day 5 of in vitro culture compared to 10% cyclic strain and non-conditioned 0% static controls. The goal of this study was to assess the effects of two modalities of tensile strain on the early phase of angiogenesis by vascular morphometric analysis in vascularized tissue constructs.

METHODS: *MVF Isolation and Seeding:* Microvascular fragments were harvested as previously described.[2] Briefly, adipose tissue was harvested from the epididymal fat pads of Lewis male retired breeder rats approved by the University of Oregon's Institutional Animal Care and Use Committee. Tissue was minced and digested with collagenase (CLS-1, Worthington), and MVFs between 20-200µm were isolated. MVFs were seeded at 40K/mL in a 3% w/v collagen type-I (Corning) hydrogel, buffered with 4X DMEM. MVF-seeded gel solutions were cast in individual, chilled (4ºC) stainless-steel apparatuses and given 45 minutes for gelation in 37ºC. This generated cylindrical constructs (5mm high, 5mm Ø) anchored between two stainless-steel meshes (40x40 mesh, 0.0185" opening, McMaster Carr) at the top and bottom of each construct. *In Vitro Tensile Strain Conditioning:* Each apparatus was attached to a displacement-controlled instrument (ElectroForce 5500, TA Instruments) and incubated in a sterile acrylic chamber as previously described. [2] Constructs that were held at 0% static strain were considered controls (n=11). In two subsequent experiments, constructs were first zeroed and preconditioned for 10 cycles at 0.05Hz from 0% to 10% tensile strain and then conditioned for 8 hours per day in either 10% static strain or 10% cyclic strain at 0.8Hz (n=5-6 per group) that corresponded to 0.5mm stretch in the MVF seeded constructs. Over the course of culture, 3D microvascular constructs were submerged in serum-free medium supplemented with Sato components [4] and recombinant human VEGF (10ng/ml; R&D Systems). On day 3 of culture, media was changed prior to the day-of tensile strain conditioning. *Vascular Morphological Assessment:* On day 5, the stainless steel meshes in each assembly were gently removed by forceps. Constructs that did not rupture were recovered and kept hydrated in 1X PBS (Gibco) prior to fixation and staining via rhodamine-labeled Griffonia simplicifolia (GS-1) lectin (Vector Labs, 10µg/ml). Intact constructs were imaged at 3 randomly selected fields using a Spinning Disk confocal microscope (Nikon SoRa) at 10X (250µm z-stack, 5µm step size). Each confocal z-stack was 3D median filtered and deconvolved before volumetric segmentation and network morphological assessment to measure branching and total network length as previously described (AMIRA) [5]. Data are shown as mean ± standard deviation. Kruskal-Wallis with Dunn's multiple comparisons test (GraphPad Prism 10, α=0.05) was used to compare vascular network morphometrics. Any outliers were identified and removed using the ROUT method (Q=1%).

RESULTS SECTION: Lectin images qualitatively revealed inhibited vascular network formation due to 10% cyclic tensile strain (Fig. 1A). The mean total network length was 253% higher in MVF-seeded constructs that were conditioned in 10% static tensile strain (24,699± 6920 µm) as compared to 10% cyclic strain (9,780±1571 µm) (p= 0.0185) (Fig. 1B). While there were no differences in network length and branching between 0% static controls and 10% tensile strain conditioning groups, there was a significant increase in the number of branch points due to 10% static strain (84.89±30.53) compared to 10% cyclic strain (25.55±5.681) (p=0.0218).

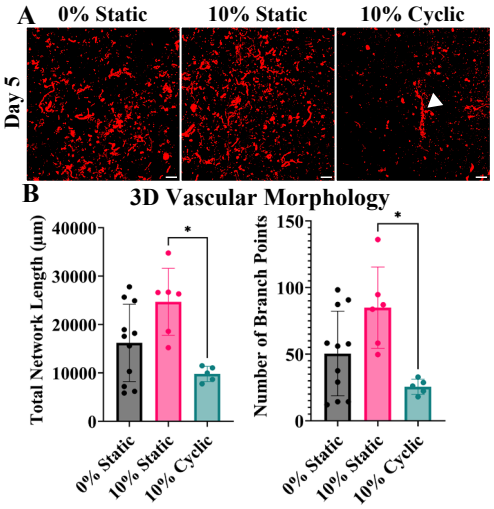

Figure 1: A) Representative images; Scale bar = 150 µm. White arrow designates destabilized vessel. B) Volumetric morphological quantification of neovascularization; *p-value < 0.05.

DISCUSSION: In this work, we assessed two modes of tensile strain conditioning and their effects on vascular morphology at day 5 of *in vitro* culture. Static tensile strain for 8 hours each day enhanced day 5 network length and branching number compared to cyclic strain. This is consistent with similar benefits due to mechanical stretch *in vivo* and its effects on microvascular density. [6] However, our conditioning behaviors did not significantly affect the vascular morphology when compared to our static controls. This suggests that the early presentation of tensile strain during the first phase of angiogenesis may not provide a beneficial cue for angiogenesis, which has been similarly identified during the early application of compressive strain. [2] Future work will explore additional magnitudes, frequencies, and the temporal relationship of tensile strain on angiogenesis in our novel 3D tensile strain *in vitro* model system. This work will aid in identification of the advantageous and detrimental windows of mechanically stimulated vascular growth. Future characterization of vascular alignment within these constructs will further enhance our understanding of the effects of tensile strain conditioning on vascular morphology.

SIGNIFICANCE/CLINICAL RELEVANCE: This work provides insight in how static and cyclic tensile strain conditioning affects vascular growth during the early phase of angiogenesis *in vitro*. These principles will be utilized in future work with multi-tissue and patient specific organoid models that recapitulate aspects of musculoskeletal tissue injury and adaptation to help inform regenerative rehabilitation treatment protocols.

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ACKNOWLEDGEMENTS: This work was supported by the Wu Tsai Human Performance Alliance and the Joe and Clara Tsai Foundation.