Revealing Spatial Cellular Responsivity in Fiber-Reinforced Micro-Environments for Meniscus Tissue Engineering

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Introduction: The crucial role of the menisci in load distribution and lubrication of the knee renders the tissues highly susceptible to damage. To replicate the aligned nature of the meniscus via tissue engineering, several advances have been made in fiber-reinforcement technology for recapitulation of the circumferentially-oriented, load-bearing properties of the tissue [1,2]. Generally, these strategies involve encapsulation of cells within a natural soft hydrogel or scaffold reinforced by a stiff polymer fiber network. However, these studies focus on macro-scale aspects of these scaffolds, neglecting to consider the micro-scale cell-biomaterial interactions that govern remodeling and ECM deposition/organization towards an aligned neo-tissue. In fact, during development, cell alignment precedes aligned tissue deposition [3], highlighting the need to induce early cell response. Since the spatial response of cells within a fiber-reinforced micro-environment likely influences long-term efficacy of meniscal replacements, the objective of this research was to elucidate patterns of cell response within these micro-environments. We utilized cell shape and mechano-response parameters in large heterogenous data sets using machine-learning strategies [4] to better uncover these trends.

Methods: To simulate a fiber-reinforced microenvironment, poly(glycolide-co-caprolactone) (PGCL) fiber was embedded within fibrin gels containing marrow-derived cells. Constructs were cultured for 3 days in chemically defined media supplemented with 10 ng/ml TGF-β3 and varying concentrations of aprotinin (anti-fibrinolytic to tune remodeling rate). Following culture, cells were stained with DAPI, Phalloidin, and YAP1 to visualize cell shape and mechano-response. Cell and nuclear shape parameters, YAP nuclear ratio, and distance from fiber were measured for individual cells (Fig 1A, Fig 1B). Principal component analysis (PCA) was performed to project 23 input parameters (not including distance) onto 5 principal components (PCs), upon which Agglomerative Hierarchical Clustering (AHC) was applied to cluster cells into 3 groups.

Results: Within fiber-reinforced fibrin constructs, the total set of cells showed a negative trend between YAP nuclear localization and distance from the fiber. However, this correlation was weak (R^2=0.0554), illustrating the cellular heterogeneity within the micro-environment (Fig 1C). Following PCA and AHC, cells were clustered into 3 groups (Fig 1D). By analyzing individual cell clusters, significant differences in YAP nuclear localization were shown between clusters (Fig 2A). Consequently, these clusters were labeled as High Response (HR), Medium Response (MR), and Low Response (LR) based on YAP nuclear localization. Spatial patterns between clusters were evident, with a general trend from HR to MR to LR with increasing distance from fiber (Fig 2B, Fig 2C). Finally, modulation of fibrin remodeling capacity, controlled by varying Aprotinin concentrations, showed a higher dosage of Aprotinin (100 KIU/ml) led to HR cells being present closer to the fiber (Fig 3A), while less Aprotinin (10 KIU/ml) led to higher YAP localization in HR and MR cells (Fig 3B).

Discussion: This study demonstrated patterns of spatial cell responsivity in fiber-reinforced microenvironments, based on cell and nuclear parameters that were used to cluster cells using a PCA-AHC approach. Cell responsivity within this micro-environment was influenced by the spatial localization of individual cells around a stiffer polymeric fiber. Furthermore, modulation of the fibrin remodeling capacity (low vs high Aprotinin) further influenced the level of responsiveness and localization of certain cell clusters around the fiber, which may foreshadow longer-term aligned tissue deposition.

Significance: A clustering approach to manipulate a large, heterogeneous set of cells can be used to reveal patterns of spatial cellular responsivity in fiber-reinforced scaffold environments in order to optimize micro-scale fabrication methods of stiff-soft composite scaffolds for meniscus replacement.

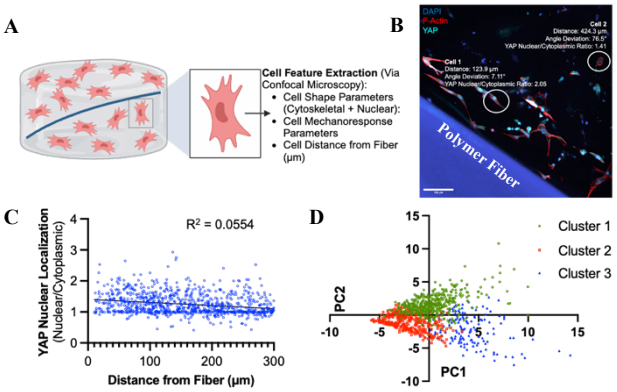

Figure 1. *[A] Cell parameters measured for individual cells (23 measurements were used for Principal Component Analysis). [B] Distance, aspect ratio, and YAP Nuclear/Cytoplasmic ratio for two individual cells, one near and one far from a polymeric fiber (in blue). [C] YAP nuclear localization of individual cells vs. distance from fiber (μm) (n=943; R^2=0.0554) [D] Principal Component 1 vs. Principal Component 2 colored by cluster following Agglomerative Hierarchical Clustering.*

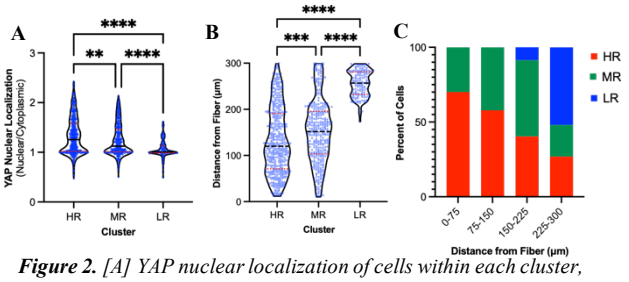

Figure 2. *[A] YAP nuclear localization of cells within each cluster, labeled as: HR=High Response (n=450), MR = Medium Response (n=351), LR=Low Response (n=142) [B] Distance from fiber (μm) of individual cells from each cluster. [C] Percent of each cell cluster localized within distance bins from fiber. **,***, **** represent p<0.01, 0.001, 0.0001, respectively.*

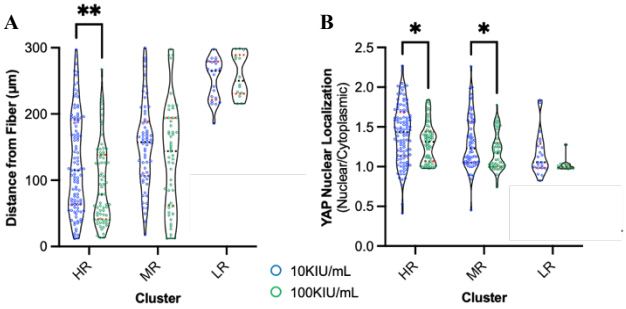

Figure 3. *[A] Distance from fiber (μm) and [B] YAP nuclear localization of cells within fiber-reinforced constructs, cultured with 10 KIU/ml and 100 KIU/ml aprotinin, grouped by cell cluster. *, ** represent p<0.05, 0.01, respectively.*

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References: [1] Patel+ *Am J Sports Med.* (2018). [2] Lee+ *Sci Transl Med*. (2014). [3] Tsinman+ *FASEB J*. (2021). [4] Bonnevie+ *Sci Rep*. (2021).