Thy-1 negative fibroblasts are an immuno-responsive subpopulation critical for biomaterial-mediated fibrosis

Daniel Abebayehu[1], Grace Bingham[1], Drew Miller[1], Donald Griffin[1], Thomas H. Barker[1]
[1]Department of Biomedical Engineering, University of Virginia

Statement of Purpose: Implanted biomaterials are critical for numerous clinical applications and emerging therapies. A common barrier to success is evading the foreign body response and fibrotic encapsulation of these implants. Recent work in other fibrotic disorders has characterized that fibroblasts lacking Thy-1, a GPI-anchored membrane protein that serves as a rigidity sensing molecule, are the subpopulation of fibroblasts present at the active sites of fibrosis (Hagood JS. Am J Pathol, 2005; 167(2): 365-79). It is currently unknown whether Thy-1 plays a role in biomaterial-mediated fibrosis and what promotes the loss of Thy-1. Leveraging a hydrogel system that has been extensively shown to be regenerative (Griffin DR. Nat Mater 2015; 14(7): 737-44) and comparing to a fibrotic hydrogel of similar chemistry, we hypothesize that regenerative biomaterials can become fibrotic in the absence of Thy-1 and that the loss of Thy-1 is mediated via chronic inflammatory signaling.

Methods: Using WT and Thy-1 KO mice, we subcutaneously implanted microporous annealed particle (MAP) hydrogels made from PEG and compared them to standard bulk nanoporous PEG hydrogels. To identify cytokines for our study, we first mined publicly available datasets and identified all known cytokine receptors expressed on fibroblasts. Being careful to not artificially mechanically stimulate our cells, CCL-210 human fibroblasts were treated on physiologically soft (~2kPa) substrates with the cytokines that correspond to the identified receptors. We used flow cytometry to measure the loss of Thy-1, the dynamics of that loss, and whether this cytokine-induced loss recapitulates the Thy-1 negative myofibroblastic phenotype seen in disease. Based on observed heterogeneity, we used single cell RNA-Sequencing (scRNA-Seq) to identify the subpopulations that emerge in response to cytokine treatment and used the Seurat toolkit to perform the scRNA-Seq analysis.

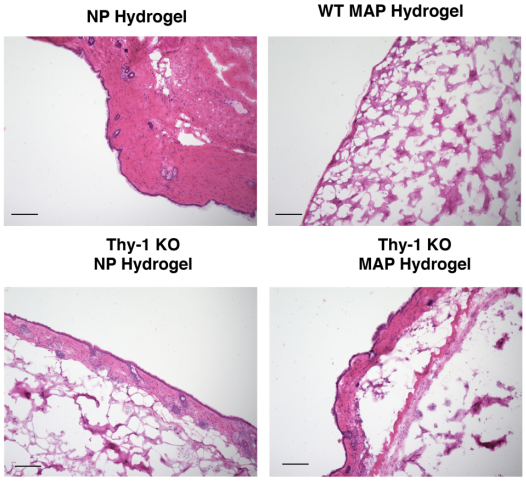

Fig 1. Subcutaneous implantation of nanoporous and MAP poly-ethylene glycol gels

Results: As previously shown in WT mice, nanoporous hydrogels elicited fibrotic encapsulation and foreign body giant cells, whereas MAP hydrogels had little to no fibrotic capsule and demonstrated better cell infiltration. However, when implanted in Thy-1 KO mice, both the nanoporous and MAP hydrogels produced a fibrotic capsule and foreign body giant cell formation that was indistinguishable from one another. Based on elevated NFκB activation in the fibrotic implants, we used IL-1β and TNFα on CCL-210, which prompted the emergence of Thy-1 negative subpopulations at approximately 25%. IL-1β and TNFα both induced aberrant fibroblast spreading and a unique αSMA-expressing fibroblast subpopulation. Furthermore, fibroblasts that were sorted to be Thy-1 positive after cytokine treatment were resistant to Thy-1 loss, indicating a heterogeneity that needed to be characterized. scRNA-Seq analysis of IL-1β and TNFα treated fibroblasts illustrated the emergence of 2 new subpopulations and the expansion of another upon cytokine stimulation, all characterized by an "immuno" phenotype. Strikingly, all three subpopulations are characterized by the loss of Thy-1 transcription, in stark contrast to all endogenous/unstimulated human lung fibroblast. Interestingly, one of the markers identified among these inflammatory fibroblasts, IL1R1, is expressed among the cells within the fibrotic capsule of nanoporous hydrogels (in WT and Thy-1 KO) and MAP gels in Thy-1 KO mice. This work strongly suggests the existence of a nascent fibroblast subpopulation that is uniquely prone to immune-induction of an aberrantly mechanotransductive, pro-myofibroblastic phenotype. Past studies by others have demonstrated that Thy-1 negative fibroblasts are highly resistant to apoptosis and thus this immuno-stromal axis represents a plausible link between inflammatory signaling and fibrotic responses that can help eliminate biomaterial-mediated fibrosis.

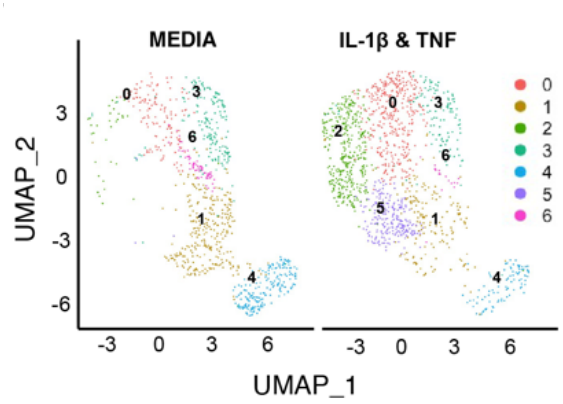

Fig 2. Changes in fibroblast heterogeneity in response to cytokines.