Optimizing the ECM Pattern of 2D Tissues for the Study of Pulmonary Fibrosis

Background

•Approximately 5 million individuals worldwide suffer from Idiopathic Pulmonary Fibrosis (IPF), an interstitial lung disorder, which causes progressive scarring of lung tissue that is generally irreversible and affects the ability of get enough oxygen into the bloodstream.

•To better understand the mechanisms of IPF it is necessary to understand the contractile forces in fibrotic lung cells in relation to that of healthy lung cells.

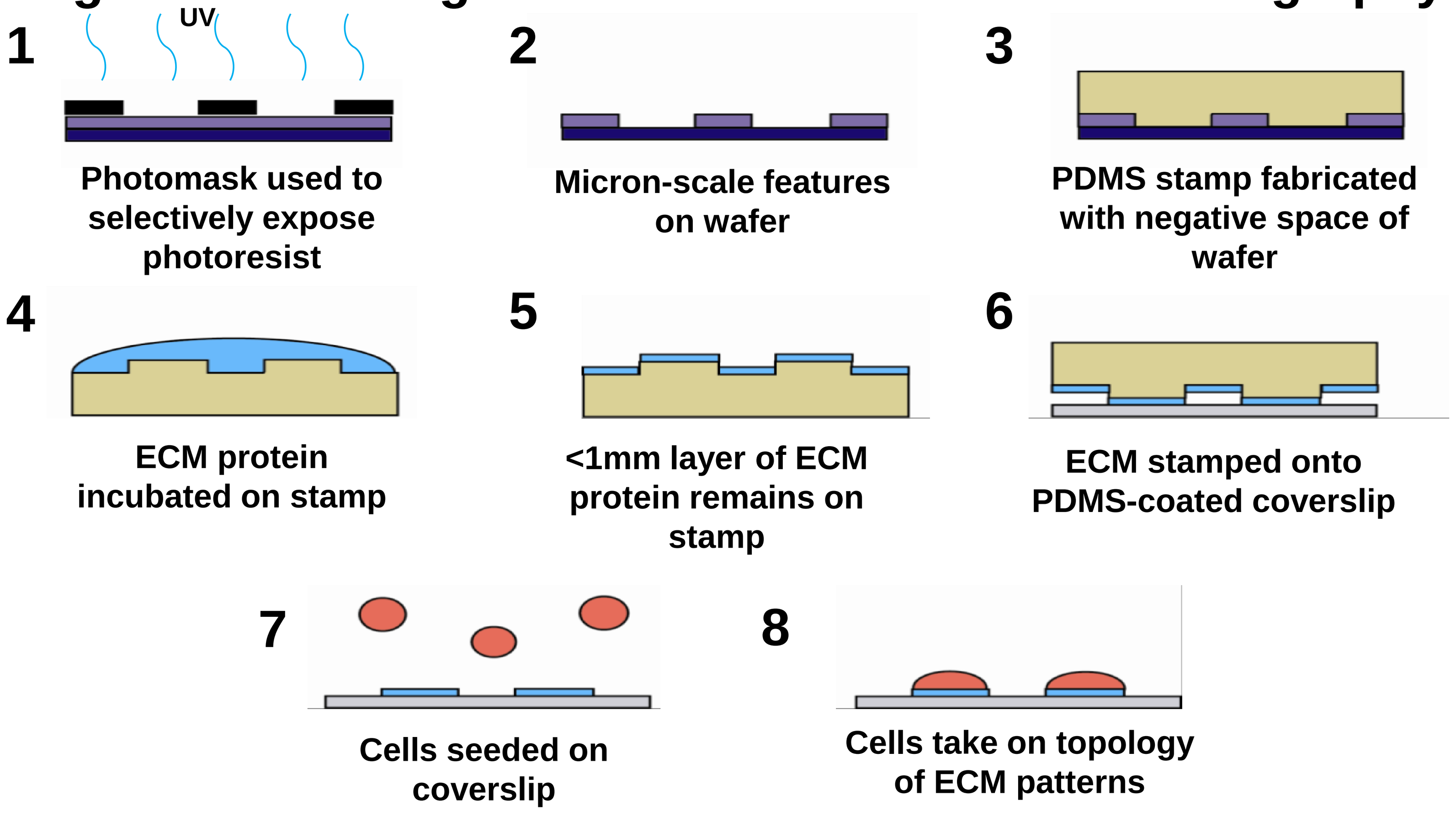
•Professor Patrick Alford uses a microfabricated thin film technique that he developed to analyze the cell traction forces in engineered tissus in which he constructs a 2-D engineered tissue on a flexible polymer beam, whose curvature can then be used to calculate the cell traction forces from the engineered tissue.

•For the thin film method to work properly the tissue must be highly aligned and confluent.

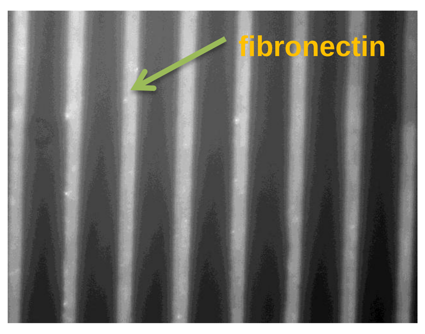
•My research involved finding the extra cellular micro-pattern that optimizes tissue alignment and confluence for measurements of traction forces of fibrotic lung cells.

Methods

•**Figure 1: Building Vascular Muscle with Soft Lithography**



•**Figure 2: Fluorescence stained ECM on PDMS-coated Coverslip. The bright regions contain fibronectin while the darker regions contain unstained PDMS.**



•5 stamping patterns were used: 5X5, 10X5, 10X10, and 20X5 ECM band width X gap width(μm) patterns, as well as a flush ECM coating pattern.

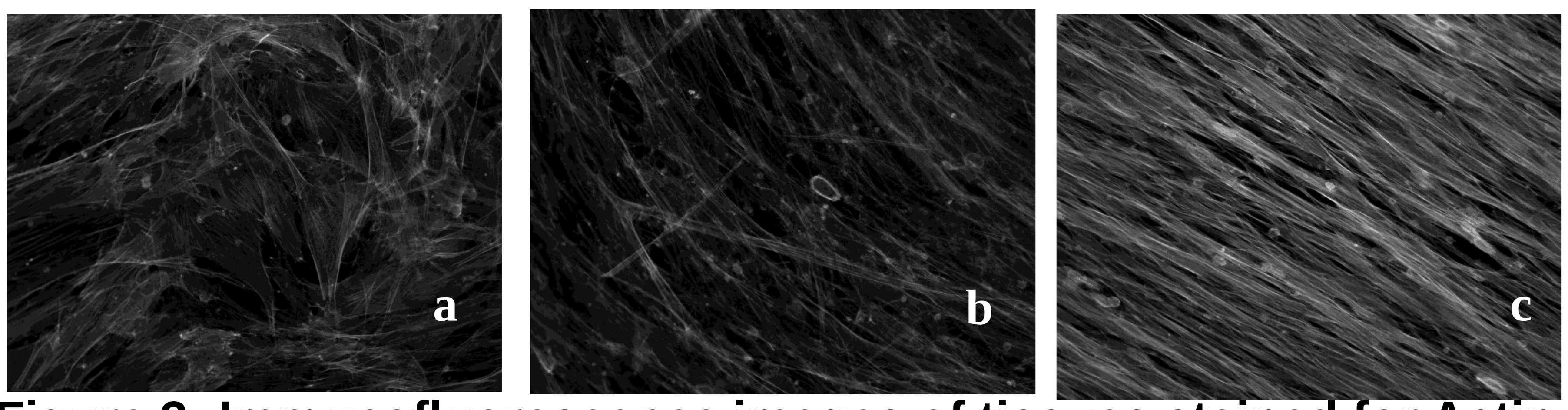


Figure 3: Immunofluorescence images of tissues stained for Actin Thin Filaments

- Idiopathic Pulmonary Fibroblasts (IPF cells) and Human Pulmonary Fibroblasts (HPF cells) were seeded at least 4 times on each ECM pattern.
- Once seeded and confluent, the engineered tissues were then fixed and stained with immunofluorescent dyes.
- Immunofluorescent imaging and quantitative analysis tools developed in the Alford Lab were then used to calculate the Orientational Order Parameter (OOP), a proxy for allignment
- Images a and b are of tissues engineered using the flush ECM pattern and had OOP's of .22 and .74 respectively. Image c is of a tissue engineered using the 5X5 ECM pattern and had an OOP of .97.

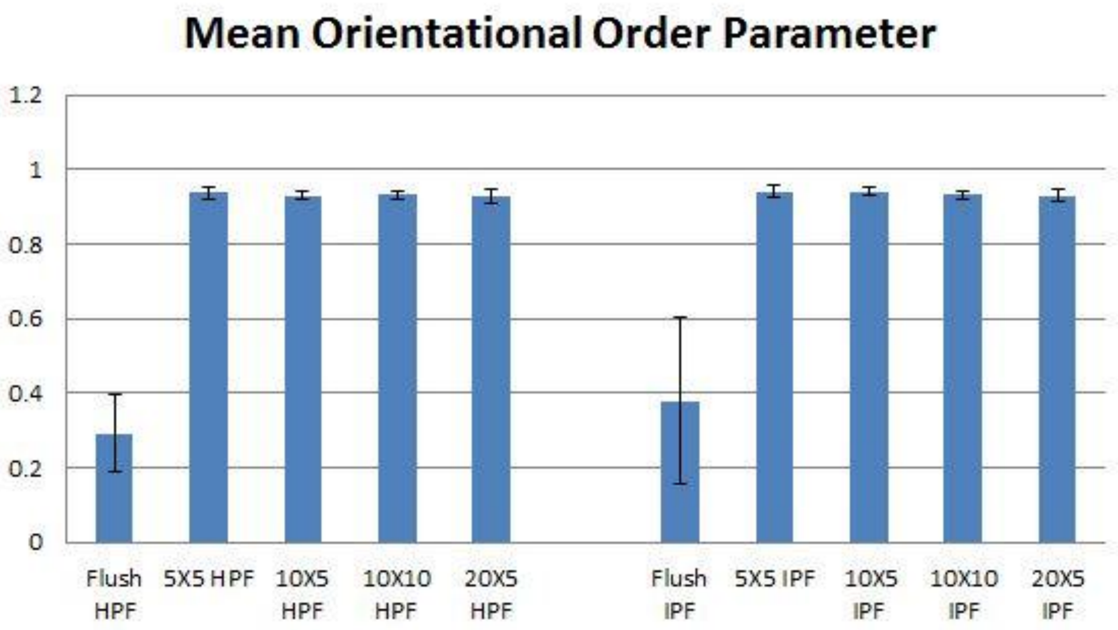
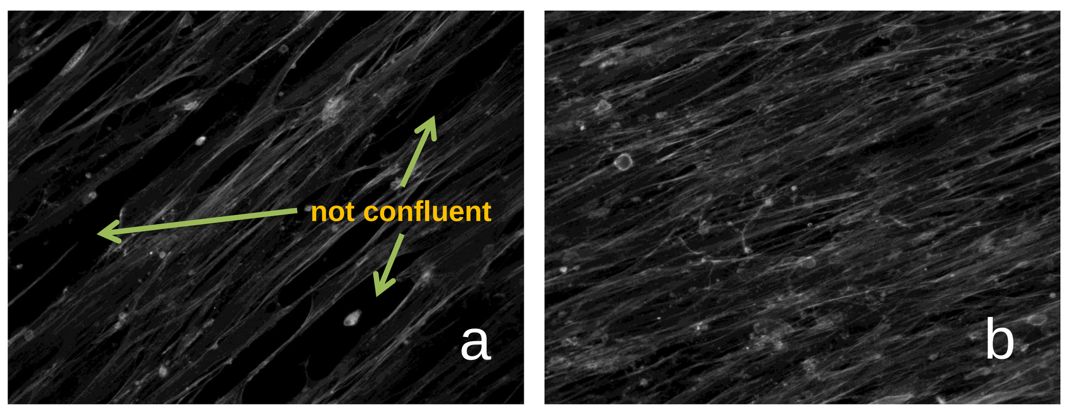


Figure 4: Mean Orientational Order Parameter values calculated as a measure of alignment for each engineered tissue type.

- For the linear ECM patterns a standard deviation of .018 or less was seen in the OOP values for each tissue type.

Figure 5: Confluence



- Though they displayed OOP's averaging .93 for both cell types, the tissues engineered using the 10X10 ECM pattern were not confluent due to the large gaps between ECM portions, as can be seen in image a.
- Image b, is an example of a confluent tissue that was engineered using the 5X5 ECM pattern and IPF cells.

Discussion

- Though the tissues engineered with the flush ECM pattern were confluent, their OOP values averaging less than .4 show that they are not highly aligned, and therefore not optimal for further studies.
- Additionally, though the tissues engineered using the 10X10 ECM pattern were highly aligned, with OOP's averaging .93, they do not provide value for further research because of their lack of confluence.
- Because the optimal engineered tissue for the study of contractile forces in fibroblasts is both highly aligned and confluent, the tissues engineered using the 5X5, 10X5, and 20X5 ECM patterns remain as strong candidates for use in further research.