Figure S1



Figure 1. Decellularized IPF lungs display decreased elasticity. (A) Human lung samples were decellularized and Masson's Trichrome and picrosirius red images are shown for control and IPF matrix. (B) Mechanical properties of decellularized lung were measured using an Instron5848 with a 10N load by cross-head displacement and stress - strain plots are generated with (C) the Young's Moduli calculated at 20% strain. (individual points plotted with mean \pm SEM, * p<0.05 compared to control lung ECM, N=8-9).



Figure 2. Flow cytometry characterization of cultured PC isolated from human placental microvessels. Flow cytometry plots demonstrating that PC express the cell surface molecules NG2, CD90, PDGFR- β , and CD146. PC do not express the intracellular proteins SMMHC, a marker expressed by smooth muscle cells, CD31 or CD34, markers expressed by endothelial cells, or CD45, a marker expressed by leukocytes. Specific staining is shown by the bolded black line whereas isotype control is shown in grey. Representative plots are shown for pericyte isolations from N > 5 individual human donors. (N=5)



Figure 3. Polyacrylamide hydrogel evaluation. Polyacrylamide substrates of varying stiffness were made by altering the relative concentration of crosslinker. (**A**) Representative images of the low, medium and high stiffness hydrogels are shown on a custom made apparatus with a 15mg sphere placed on the center. (**B**) Young's Modulus of low, medium, and high stiffness hydrogels were measured using a modified model of the Hertz equation (* p<0.05 compared to low stiffness hydrogel, # p<0.05 compared to medium stiffness hydrogel, N>4). (**C**) Healthy and IPF lung matrices were solubilized and conjugated to the polyacrylamide hydrogels and the Young's Moduli were calculated at 20% strain using an Instron 5848 with a 10N load cell by cross-head displacement (* p<0.05 compared to low stiffness hydrogel of the same ECM, # p<0.05 compared to medium stiffness hydrogel of the same ECM, N>5). (**D**) To confirm conjugation of ECM to the polyacrylamide hydrogels, the ECM was tagged with NHS-FITC, dialyzed, and conjugated to the polyacrylamide hydrogel. The excess ECM-FITC was removed by rinsing and fluorescent images were taken for control, 0.2 mg/mL (collagen I and fibronectin), and 0.5 mg/mL of both the healthy and IPF lung ECM.





Figure 4. Varying matrix content does not alter PC to expression of \alpha-SMA. (A) Human fibronectin containing the EDA fragment was isolated from pericyte culture condition media using gelatin affinity chromatography. Immunoblotting using an EDA-FN specific antibody (IST-9) confirmed the presence of EDA-FN, but did not bind to plasma fibronectin. (B-C) Two concentrations of EDA-FN and collagen I (0.1mg/mL and 0.5mg/mL) were conjugated to hydrogels of low, medium, and high stiffness. PC were cultured on the hydrogels for 7 days and α -SMA expression was quantified using flow cytometry analysis for hydrogels conjugated to EDA-FN and collagen I (* p<0.05 compared to low stiffness hydrogel conjugated to 0.1mg/mL of the corresponding protein, N=3). (D) No significant difference was seen between ECM concentrations. Images of PC on the hydrogels at 7 days are shown. PC were cultured on hydrogels of low, medium, and high stiffness functionalized with a 1:1 ratio of fibronectin and collagen I. (* p<0.05 versus low stiffness hydrogel, # p<0.05 versus medium stiffness hydrogel, N=4).