Appendix for the paper:

Title: Computational Modeling predicts simultaneous targeting of fibroblasts and epithelial cells is necessary for successful treatment of pulmonary fibrosis

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Multi-Scale Model Rules

Here we describe the overall structure of our multi-scale hybrid agent-based model of a fibroblast/epithelial cell co-culture system. There are two key models that are linked to create the multi-scale model: *a cellular scale* model and a *molecular scale* model. Here we include a detailed description of the stochastic processes occurring in the cellular scale model that reads out at a tissue scale. The discrete molecular scale processes are described in full detail in a previous study (1). A schematic capturing how these models were derived independently and are linked is included in the main text (Figure 2) along with a description of how the model linking is done (see Methods). All values and definitions of parameter that are used in the models are listed in the Supplementary Tables S1, S2, S3, S4.

Overall Structure of the Cellular Scale ABM

Agent based models are designed focusing on four components as follows: an environment (simulated tissue culture plate), agents (fibroblasts, myofibroblasts, and epithelial cells), rules that govern agent behavior, and a time-step that dictates how frequently events in the model are updated (Δt).

Environment: The three-dimensional environment in this model represents one tenth of a well in a 96 well tissue culture plate. At the bottom of the model environment is a square surface measuring 1.78mm x 1.78mm. This surface represents the plastic at the bottom of a dish. Above this layer is a three-dimensional layer that represents the volume of the plate that is divided into a 6561 compartment grid (81 compartments by 81 compartments). Each compartment is a 22µm x 22µm x 22µm cube. Cellular agents are adherent to the "plastic" and are therefore restricted to the grid compartments directly above the *in silico* plastic bottom surface in what will be defined as the cellular layer. Because cells are adhesion dependent, cells can only move in two dimensions within the cellular layer. We assume that each grid compartment can contain only one agent at a time based on their size. Their movement is guided by the ABM rules defined below. The full 3D model contains 282 layers each with 6561 compartments. All layers above the cellular layer represent *in silico* media in the well of a tissue culture plate.

Some mediators are restricted to the two-dimensional (2D) bottom surface layer while others can diffuse throughout the three dimensional compartments. Extra-cellular matrix (ECM) proteins are secreted directly only the surface of the virtual dish where they are cross-linked into a matrix. These proteins accumulate on this layer and do not diffuse. Latent TGF-β1 (as secreted by fibroblasts and myofibroblasts) is secreted into the extracellular matrix where it adheres until the protein is activated. Proteins that do not diffuse are stored as continuous values in the compartment where they are secreted. Activated TGF-β1 and PGE_2 are soluble mediators which can diffuse in three dimensions throughout the entire volume of the model, including the cellular layer and all media layers above it. These mediators are also stored as continuous values that occupy space anywhere within the model.

We built an 81 x 81 x 282 3D grid. We assume there are no flux boundary conditions for all boundaries of the model grid for all agent movement and molecular diffusion steps. 5000 epithelial cells and 500 fibroblasts are randomly placed on the grid, each within its own compartment in the cellular layer. This is consistent with *in vitro* studies typically plating a 10:1 ratio of epithelial cell to fibroblasts (2).

Agents*:* There are two distinct types of cellular agents in the model, fibroblasts (representing pulmonary fibroblasts) and epithelial cells (representing alveolar epithelial cells). We assume that fibroblasts can differentiate into myofibroblasts, a process that is driven by αSMA synthesis (see Methods in main text). We also assume they do not de-differentiate back into a fibroblast, but rather remain a myofibroblast for the duration of a simulation. Once differentiated, we assume that

myofibroblasts can no longer move or proliferate. Myofibroblasts are also susceptible to undergoing apoptosis in a probabilistic fashion.

Cellular Scale ABM Rules: ABM rules define cellular interactions as well as other processes in the model including cell movement, proliferation, death, and mediator secretion according to known biological behaviors in vitro. Additional rules in the model define molecule diffusion and degradation based on known rates identified in the literature (see Parameters below).

1. Cell Movement. We assume that fibroblasts are the only cellular agents with the ability to move. They can move in 8 possible directions within the cellular layer. Fibroblasts examine their Moore neighborhood (the nine grid compartments surrounding the cell including the compartment occupied by the cell) and determine whether there is an unoccupied compartment. If an unoccupied compartment is found, then the cell has a probability of moving into that compartment (Table S2). A fibroblast can move up to one time during a model time step.

2. Cell death, proliferation and differentiation*. A.* *Cell death due to age.* Two agent types, myofibroblasts and epithelial cells, can die of old age. When a cell reaches its maximum allowable age there is a probability that the cell will die. Dead cells will be removed from the grid (Table S1, S3). ***B.*** *Epithelial cell death due to TGF-β1 concentration.* Epithelial cells are sensitive to high concentrations of TGF-β1. At birth each epithelial cell is assigned a maximum TGF-β1 threshold from a uniform distribution. This threshold captures the idea that that each epithelial cell has a slightly different sensitivity to TGF-β1 (3). At each model time step, epithelial cells checks the cumulative amount of TGF-β1 that it has bound. If the cumulative bound TGF-β1 reaches or exceeds the cells maximum threshold, then the cell dies and is removed from the grid. ***C.*** *Fibroblast proliferation.* Four rules govern when a fibroblast can proliferate. (1) It must be 24 hours since the cell last divided (4). (2) The cell must have bound enough TGF-β1 to meet the TGF-β1 proliferation threshold (Table S1). (3) The cell must not have bound more than the maximum amount of PGE_2 permissive of proliferation (Table S1). (4) There must be an empty compartment in the fibroblasts Moore neighborhood for a daughter cell to enter. If these four conditions are met, then the fibroblast has a non-zero probability of proliferating (Table S1). ***D.*** *Fibroblast to myofibroblast differentiation.* We assume that fibroblasts to myofibroblast differentiation is determined by the amount of αSMA synthesized by a fibroblast and is described in detail in the "Multi-scale Model Calibration" section of the main text.

3. Mediator Secretion. We assume that fibroblasts are able to secrete both TGF-β1 and PGE_2 at rates listed in Table S1, respectively. We also assume that epithelial cells secrete only PGE_2 at rates listed in Table S1. Finally, we assume that myofibroblasts secrete TGF-β1 and ECM proteins with rates listed in Table S3.

4. Diffusion and degradation. Continuous molecular diffusion is calculated in three dimensions using the Fast Fourier Transform method (5). To integrate this numerical method into the framework of an ABM we use the methods described in detail by Cilfone et al. 2014 (6).

Time step: Interactions between agents are updated for each ABM time step (Δt = 1 hour) and are described above. Molecular scale processes, specifically TGF-β1 receptor ligand signaling in fibroblasts and secretion of mediators by all agent types, generally occur more quickly than cellular scale processes. For example, rates of cell proliferation are about 24 hours (7, 8) where rates of TGF-β1 receptor synthesis are around 4 minutes (9). These molecular processes are therefore updated on a more frequent molecular time step (Δt = 10 seconds), thus each molecular process is updated 360 times per ABM time step. Diffusion of soluble molecules is updated on an additional diffusion time step (Δt = 60 seconds).

Multi-Scale Model Parameters

Table S1: Extracellular mediator Parameters and model simulation times

Parameter	*Definition*	*Value*	*Source*
Δt_{ABM}	Time over which the ABM is updated	1 h	N/A
$\Delta t_{diffusion}$	Time over which diffusion is solved	60 s	N/A
$\Delta t_{smoother}$	Time over which diffusion smoother is solved	0.1 s	N/A
$N_{smoother}$	Number of times diffusion smoother is solver in one diffusion time step	10	N/A
$\Delta t_{molecular}$	Time over which TGF-β1 receptor ligand molecular model is solved	10 s	N/A
$D_{TGF\beta1}$	Diffusivity of active TGF-β1	2.7×10^{-7} cm^2/s	DMW
D_{PGE2}	Diffusivity of PGE_2	5.23×10^{-6} cm^2/s	DMW
$\delta_{TGF\beta1}$	Active TGF-β1 degradation rate constant	[0.001 , 0.1] pM/min	(10)
δ_{PGE2}	PGE_2 degradation rate constant	[0.001 , 0.1] pM/min	Est.

N/A = not applicable DMW = derived from molecular weight Est. = estimated by uncertainty and sensitivity analysis

Table S2: Fibroblast Parameters

Parameter	*Definition*	*Value*	*Source*
$t_{proliferation}$	Minimum time between proliferation events	24 h	DNS
$max_{prolifPGE2}$	Maximum bound PGE_2 permissive of proliferation	[5×10^{-21}, 1×10^{-9}] pM	Est., Fig 3B

$min_{prolifTGF\beta1}$	Minimum bound TGF-β1 necessary for proliferation	[1x10^{-16}, 1x10^{-14}] pM	Fig. S
$m_{differentiation}$	Slope of the linear regression dictating fibroblast differentiation	0.5~~12~~	Est.
P_{move}	Probability of fibroblast movement	[1x10^{-2}, 1]	N/A

DNS = data not shown N/A = not applicable Est. = estimated by uncertainty and sensitivity analysis

Table S3: Myofibroblast Parameters

Parameter	*Definition*	*Value*	*Source*
V_{ECM}	Rate of ECM synthesis	[1, 100] units	Est.
min_{age}	Minimum lifespan of myofibroblasts	1440 h	(11)
P_{death}	Probability of death	[0, 1]	N/A
max_{ECM}	Maximum amount of ECM in a single compartment	100 units	N/A

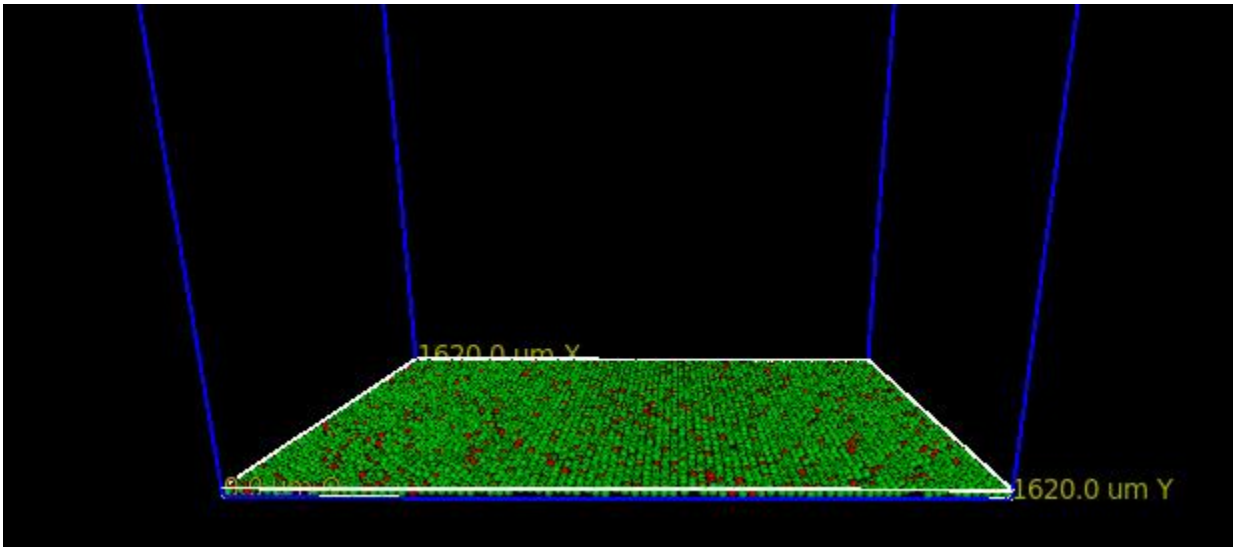
Est. = estimated by uncertainty and sensitivity analysis N/A = not applicable

Table S4: Epithelial Cell Parameters

Parameter	*Definition*	*Value*	*Source*
V_{ePGE2}	Rate of PGE2 synthesis by epithelial cells	[1x10^{-20}, 1x10^{-18}] pM/s	
k_{onE}	Rate of TGF-β1 binding by epithelial cells	[0.0009, 0.09] pM/s	Est.
k	Magnitude of PGE2 protection from TGF-β1 induced apoptosis	[0.0001, 100/0]	Est.
C	Non-zero constant	7x10^{-14}	Est.

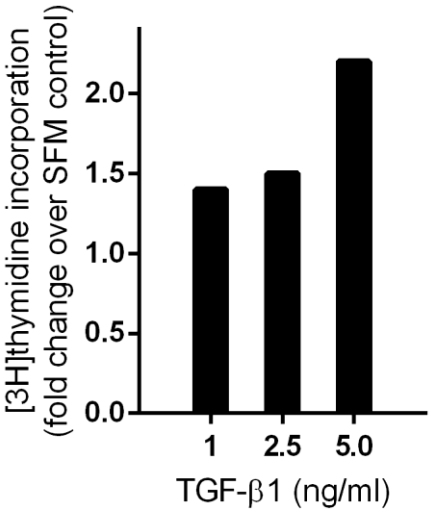
Est. = estimated by uncertainty and sensitivity analysis

Supplemental Figures



Supplemental Figure 1: Snapshot of the three-dimensional agent based model environment representing a virtual dish for the co-culture. Green spheres indicate epithelial cells while red spheres indicate fibroblasts. The blue lines indicated the edges of the boundaries of the ABM (top boundaries are not pictured) where virtual media is

present. The white lines indicate the edges of the boundaries of the cellular layer within the model (virtual plastic bottom of well).



Supplemental Figure 2: Data reproduced from Hetzel et al. 2005 showing TGF-β1 induce fibroblast proliferation (8). Normal human lung fibroblasts were cultured in either serum free media (SFM), SFM + 1.0ng/ml TGF-β1, SFM + 2.5ng/ml TGF-β1, or SFM + 5.0ng/ml TGF-β1.

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