INVENTIA LIFE SCIENCE

DATA SHEET

Fibroblast Validated Solution

3D fibroblast activation model workflow with RASTRUM

Accelerate the development of therapies targeting fibroblast activity

Part of the RASTRUM Validated Solution offering

Context of Use

This model enables users to:

- Create 3D mono-culture fibroblast models with in vivo-like network morphologies that maintain fibroblasts in a non-activated state
- Study fibroblast activation via addition of TGF-β, and assess strategies to perturb this activation status
- Develop 3D co-cultures of cancer cells and fibroblasts to model tumor microenvironment-like interactions

Model Overview

Fibroblast cells are ubiquitous and play essential roles within the tissue microenvironment, particularly through their function in extracellular matrix (ECM) remodelling. Activation of fibroblasts by growth factors, interaction with ECM, or contact with other cell types, triggers phenotypic changes that drive the production of ECM components, such as collagen. In solid cancers, fibroblast activation and consequent remodelling of ECM can modulate tumor cell biology and inhibit the efficacy of drug treatments, including immunotherapies. Therefore, given the important role of fibroblasts in tumor biology, relevant cell culture models are needed to interrogate fibroblast mechanisms of activation and ECM remodelling. Although fibroblasts can be cultured as classic 2D cell cultures, such models fail to recapitulate the in vivo ECM, and fibroblasts rapidly lose their naive features and become activated in an uncontrolled manner. These limitations can be addressed by culturing fibroblasts in 3D cell models where controlled environments do not naturally activate them.

RASTRUM matrices maintain fibroblasts in an activatable state

In RASTRUM Matrix, NHLF cells treated with TGF-β showed a marked increase in collagen I relative to untreated cells (Figure 1A). Gene expression analysis for collagen I further confirmed this difference in fibroblast activation in RASTRUM Matrices, with treated cells showing a ~4-fold increase in COL1A1 mRNA expression relative to untreated cells (Figure 1B). Collectively, the data indicate that TGF-β treatment can induce activation of fibroblasts in RASTRUM Matrix.

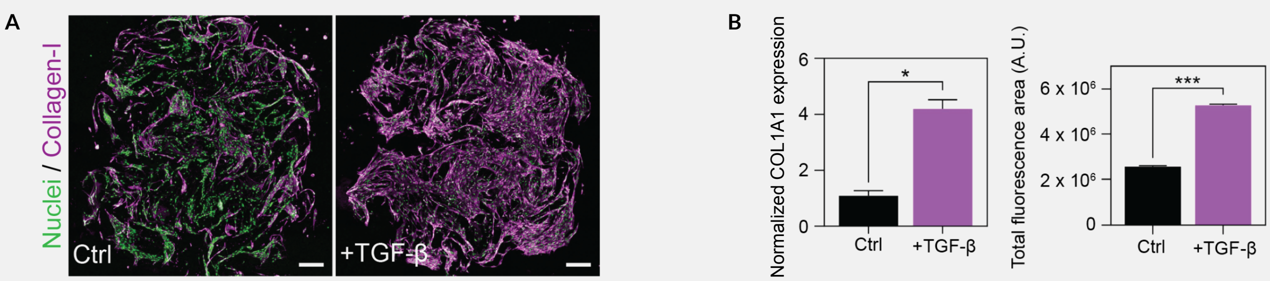

Figure 1: NHLF cells in RASTRUM Matrices produce collagen I in response to TGF-β stimulation. Representative immunofluorescence images for staining of collagen I (magenta) for untreated (ctrl) NHLF cells or TGF-β-treated NHLF cells encapsulated in RASTRUM Matrix Px02.29 in the Imaging Model at day 5 post-printing (A). Nuclear stain is Hoechst (green). Images captured at 10x magnification. Scale bars = 250 µm. (B) Increased mRNA expression of COL1A1 in NHLF cells after treatment with TGF-β (left) and image-based quantification of collagen I expression between untreated (ctrl) and TGF-β-treated cells (right). *** = P < 0.001; * = P < 0.05.

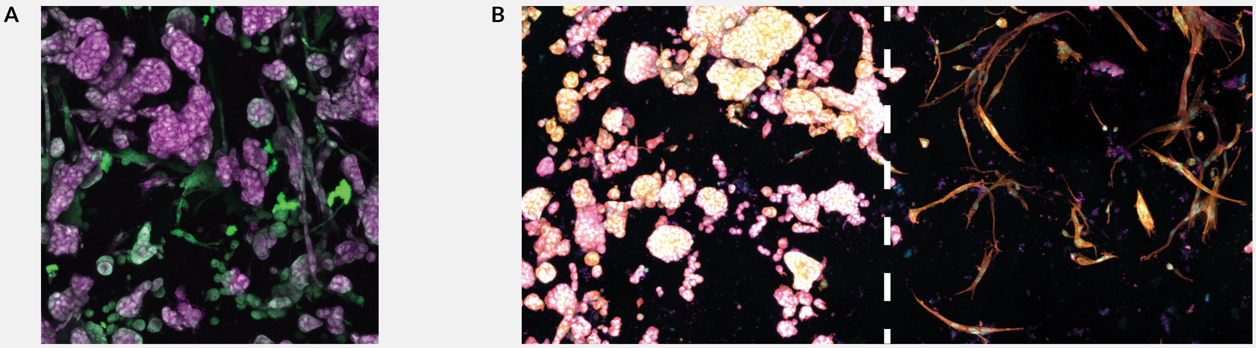

Figure 2: Fibroblast co-cultures allow modelling of tumor microenvironment-like interactions. NHLF cells cultured with A549 lung cancer cells directly in the Imaging Model (A), or co-cultured in the Dual Matrix Model (B) to allow investigation of paracrine signalling.

What this model demonstrates

- RASTRUM 3D Models maintain normal fibroblasts in an activatable state.
- Quantitative immunofluorescent imaging and gene expression analysis of markers of fibroblast activity.
- RASTRUM can be used to generate a range of 3D co-culture models containing fibroblasts and cancer cells for studying cellular crosstalk (Figure 2).

Access the full workflow documentation

- Complete protocol pack
- Reagents and materials list
- Experimental workflow guidance

Model format	Imaging Model
Matrix	Px02.29 (1.1 kPa, RGD, YIGSR, GFOGER, HA)
Cell density	NHLF: 10M/mL
Drug treatment	TGF-B day 4 post-print, 24 hr exposure

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