A Heterotypic Model of the Pancreatic Ductal Adenocarcinoma Microenvironment for Investigating Stroma-Tumor Interactions and Therapeutic Strategies

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is nearly 100% fatal. The interplay between cancer cells, stromal cells, and the collagen-rich extracellular matrix (ECM) during progression to aggressive metastasis is critical but not fully understood.[1] Greatly limiting this understanding is the lack of a PDAC model that recapitulates key features of the *in vivo* stromal microenvironment while maintaining robust physical control with imaging and therapeutic accessibility. Additionally, traditional 2D culture formats may not accurately predict *in vivo* tumor responses due to neglected stromal contributions and may therefore limit the translation of new therapeutics. We have bioengineered a microfluidic model of the PDAC stroma-cancer axis incorporating clinically-isolated multicellularity, ECM components, and a spatially-defined 3D architecture.[2] The models utility is supported by validating key features against clinically-evaluated human tissues, the ability to modulate ECM structure and monitor live cell-ECM interplay, and the potential to evaluate therapeutics in a microenvironment context.

Methods: Primary pancreatic stellate cells (PSCs) and cancer cells (PCCs) were isolated and characterized from clinically-evaluated PDAC tissue specimens. Microfluidic devices were fabricated via polydimethylsiloxane (PDMS) lithography. PSCs and PCCs, suspended in a collagen-based ECM, were patterned as 3D trilayers within devices. Void space created on each side of the trilayer was used to change media and administer therapeutic agents via capillary action. Second Harmonic Generation (SHG) imaging was used to visualize and quantify collagen structure and reorganization patterns in 111 pathology-reviewed human PDAC tissues and in different ECM formulations within the model. Microfluidic models were established, treated with increasing doses of paclitaxel for 48 hr, and assayed for cytotoxicity and culture compactness.

Results: Primary PSCs and PCCs pattern as a 3D trilayer due to laminar flow. PSC contraction of the ECM away from the PDMS sidewalls results in a compressed trilayer culture with void space flanking each side that can be used to exchange fluid via capillary action for *in situ* media changes or therapeutic administration (Fig 1).

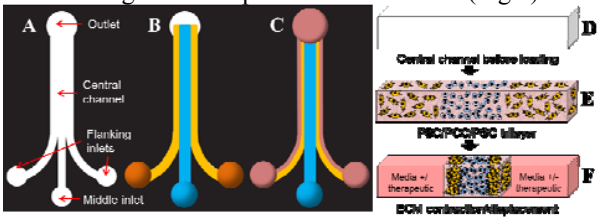

Fig 1 (A,D) Empty device (B,E) PSC-PCC-PSC trilayer in 3D ECM (C,F) contracted trilayer with media +/- therapeutics in flanking void space.

Additionally, the trilayer emulates the spatial histological relationship of dense stromal components (PSCs and collagen) encompassing disseminating cancer cells *in vivo* (Fig 2A). After ECM contraction, the interface between PSCs and PCCs can be resolved and provides a unique opportunity to parse out cell-ECM interactions as a function of cell type (Fig 2C). Compared to normal pancreas, we show that collagen fibers in PDAC tissue are significantly longer (21.02 *vs* 18.56 µm, $p<0.001$) and more aligned (0.36 *vs* 0.27, $p<0.001$). In the model, *in vivo* collagen fiber lengthening and alignment can be represented by modulating the initial ECM formulation and allowing the intrinsic contractile behavior of PSCs to remodel the collagen network (Fig 2D). This establishes a platform to study the unclear role collagen architecture has on PDAC cell behaviors. Additionally, paclitaxel treatment reduces cell viability and culture compactness in a dose-dependent manner, suggesting that both cell and ECM responses to therapeutics can be analyzed (Fig 3).

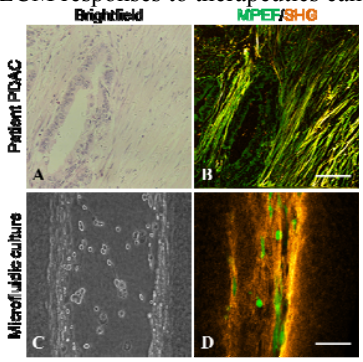

Fig 2 (A,B) *In vivo*, PCCs are surrounded by a PSC-laden stroma with linear, aligned collagen fibers. In the model (C,D), PSCs contract and align the collagen around the central PCC region. Cells are green, collagen is orange.

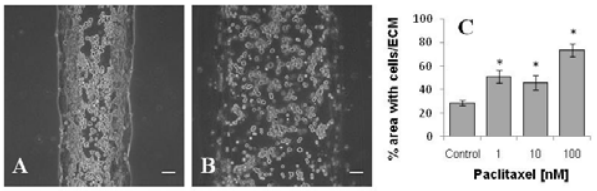

Fig 3 Trilayer culture compactness after treatment with (A) vehicle control (B) 100 nM paclitaxel. (C) Quantified cell/ECM area as an indication of trilayer compactness. Data shows mean ± SEM, * $p<0.05$ vs. control for n = 3 devices. Scale bars = 100 µm.

Conclusions: We have developed and characterized a human tissue-based PDAC model to study underlying pathological mechanisms and to assess new therapeutic approaches, which are needed to improve PDAC prognosis. The use of patient-derived tissue components, ability to introduce therapeutics, and power to modulate and quantify cell and ECM changes support the models value to PDAC translational research.

References: [1] Erkan *et al.* Nat Rev Gastroentero 2012; 9:454-67 [2] Drifka *et al.* Lab Chip 2013;13:3965-75.