Supplementary Tables

Gender	Age at surgery (yrs.)	Ethnicity	Race	Clinical diagnosis	Tumor site	Grade	Invasion	Regional lymph node	Distant metastasis	Diagnosis of tissue received from UWCCC TSB	PSC markers
F	74	NHL	C	PDAC	Body	G3	Peri-pancreatic soft tissues (pT3)	0/13 (pN0)	M0	Invasive PDAC	Vimentin (100%) αSMA (56%)
M	72	NHL	C	PDAC	Head	G2	Duodenum, bile duct (pT3)	2/14 (pN1)	M0	PDAC-associated fibrosis	Vimentin (97%) αSMA (83%)
M	76	NHL	C	PDAC	Head	G2	Duodenum (pT3)	4/22 (pN1)	M0	PDAC	Vimentin (95%) αSMA (87%)
F	62	NHL	C	PDAC	Tail	G2	Posterior peri-pancreatic adipose (pT3)	7/18 (pN1)	M0	PDAC-associated fibrosis	Vimentin (95%) αSMA (81%)

Abbreviations

F: female, M: male
NHL: not Hispanic or Latino, C: Caucasian
PDAC: pancreatic ductal adenocarcinoma
G1: well differentiated, G2: moderately differentiated, G3: poorly differentiated

Table S1 Clinical diagnosis of human tissues obtained from consented donors that underwent surgical resection for PDAC. Activated PSCs were isolated, characterized, banked, and used as part of the bioengineered PDAC microenvironment model. *In vitro* results can be correlated with histological diagnosis and disease staging.

ECM condition	ColI (mg/mL)	HA(mg/mL)	Pre-incubation temperature	Pre-incubation time
1	2	2	None	None
2	2	2	4 °C	1 hr
3	3	2	None	None
4	3	2	4 °C	1 hr
5	4	2	None	None
6	4	2	4 °C	1 hr

Table S2 Different ECM formulations used for microfluidic loading and SHG imaging.

Slide ID	Age at surgery (yrs.)	Patient diagnosis	Invasion	Regional lymph node	Distant metastasis	Tumor site	H&E slide diagnosis	Slide grading
382N1 TRIP 5-13	N/A	PDAC	Ampulla of vater and duodenum (pT3)	2/23 (pN1)	M0	Head	Normal	N/A
247N1 TRIP 5-13	N/A	PDAC	Muscularis propria layer of duodenum (pT3)	1/8 (pN1)	M0	Head	Normal	N/A
1112.1 TRIP 5-13	76	Adenocarcinoma, intestinal type	Duodenal wall (pT2)	1/23 (pN1)	M0	Intra-/peri-ampullary	Normal	N/A
7705.2	74	PDAC	Bile duct, portal vein (pT3)	2/18 (pN1)	M0	Head	100% tumor	G2
7785.2	77	PDAC	Spleen (pT3)	5/12 (pN1)	M0	Tail	80% tumor	G2
18843.2	66	PDAC	Ampulla of vater and duodenum (pT3)	4/14 (pN1)	M0	Head	2% tumor	G2

Table S3 Clinically-evaluated pancreas samples used to SHG image collagen fiber organization around intact normal and PDAC ducts.

PSC	Tissue Diagnosis	PTX IC_{50} nM
#15660.1 p-3	Fibrosis	0.0238
#15660.1 p-6	Fibrosis	0.03221
#15894.1 p-3	PDAC	0.02177
#15896.1s p-3	Fibrosis	0.0233
#15896.1s p-4	Fibrosis	0.2058
#15894.1 p-4	PDAC	0.8768

Table S4 Paclitaxel IC_{50} data for six independent 2D cytotoxicity assays on PSCs (0.19728 ± 0.34065 nM).

PANC-1	PTX IC50 nM
p-71	0.02274
p-73	0.1489
p-75	0.0238
p-76	0.02978
p-78	0.06444
p-80	0.01301

Table S5 Paclitaxel IC_{50} data for six independent 2D cytotoxicity assays on PANC-1 cells (0.05044 ± 0.05137 nM).

Supplementary Figure Captions

Fig. S1 Immunohistochemical staining of cytoskeletal markers in human normal pancreas tissue, PDAC tissue, and PSCs isolated from fresh PDAC tissue. Diffuse periacinar staining of vimentin and αSMA is observed in normal pancreas tissue. In PDAC tissue, there is an increase in αSMA (indicative of PSC activation) and loss of normal pancreatic architecture. Isolated PSCs maintained vimentin (~100%) and smooth muscle actin (80-90%) cytoskeletal markers.

Fig. S2 Collagen fiber imaging and quantification scheme around an intact normal pancreatic duct. (A) An intact normal duct is identified using a standard H&E section. (B) No filter is applied and MPE captures intrinsic cell fluorescence and eosin fluorescence (green). (C) Filtering to obtain SHG signal (orange). (D) MPE and SHG images are merged to identify the stromal-epithelial boundary of the duct. (E) The stromal-epithelial boundary is traced. (F) ctFIRE software is used to extract individual collagen fibers from the SHG image with metrics being fiber count, length, and angle. (G) CurveAlign software can then be used to analyze collagen fiber orientation relative to the stromal-epithelial boundary at a user-defined distance (green). (H) A histogram of fiber orientation angles can be generated for quantitative characterization of collagen organization around the duct. Scale bars represent 100 μm.

Fig. S3 Collagen fiber imaging and quantification scheme around an intact PDAC duct. (A) An intact normal duct is identified using a standard H&E section. (B) No filter is applied and MPE captures intrinsic cell fluorescence and eosin fluorescence (green). (C) Filtering to obtain SHG signal (orange). (D) MPE and SHG images are merged to identify the stromal-epithelial boundary of the duct. (E) The stromal-epithelial boundary is traced. (F) ctFIRE software is used to extract individual collagen fibers from the SHG image with metrics being fiber count, length,

and angle. (G) CurveAlign software can then be used to analyze collagen fiber orientation relative to the stromal-epithelial boundary at a user-defined distance (green). (H) A histogram of fiber orientation angles can be generated for quantitative characterization of collagen organization around the duct. Scale bars represent 100 μm.

Fig. S4 H&E image of G2 PDAC tissue showing disseminating epithelial cells (blue outline) surrounded by a dense PSC-laden stroma (orange arrows). Scale bar represents 100 μm.

Fig. S5 (A) Quantified fluorescence of 500 kDa FITC-dextran as it diffuses through a contracted trilayer culture after introduction through the flanking void spaces via capillary action. (B) 3D renderings of fluorescent intensity correspond to each of the timepoints.

Fig. S6 Representative brightfield microscopy images of trilayer cultures contracting as a function of time and non-epithelial cell type/density after loading. PANC-1 cell density ($1x10^6$ cells/mL) and ECM composition (2 mg/mL ColI + 2 mg/mL HA) was fixed while NHDF and PSC density was varied.

Fig. S7 Quantified ECM contraction in four trilayer culture conditions at 24, 48, and 72 hrs after loading. Each data point represents the mean ± standard deviation for three independent cultures.

Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Figures

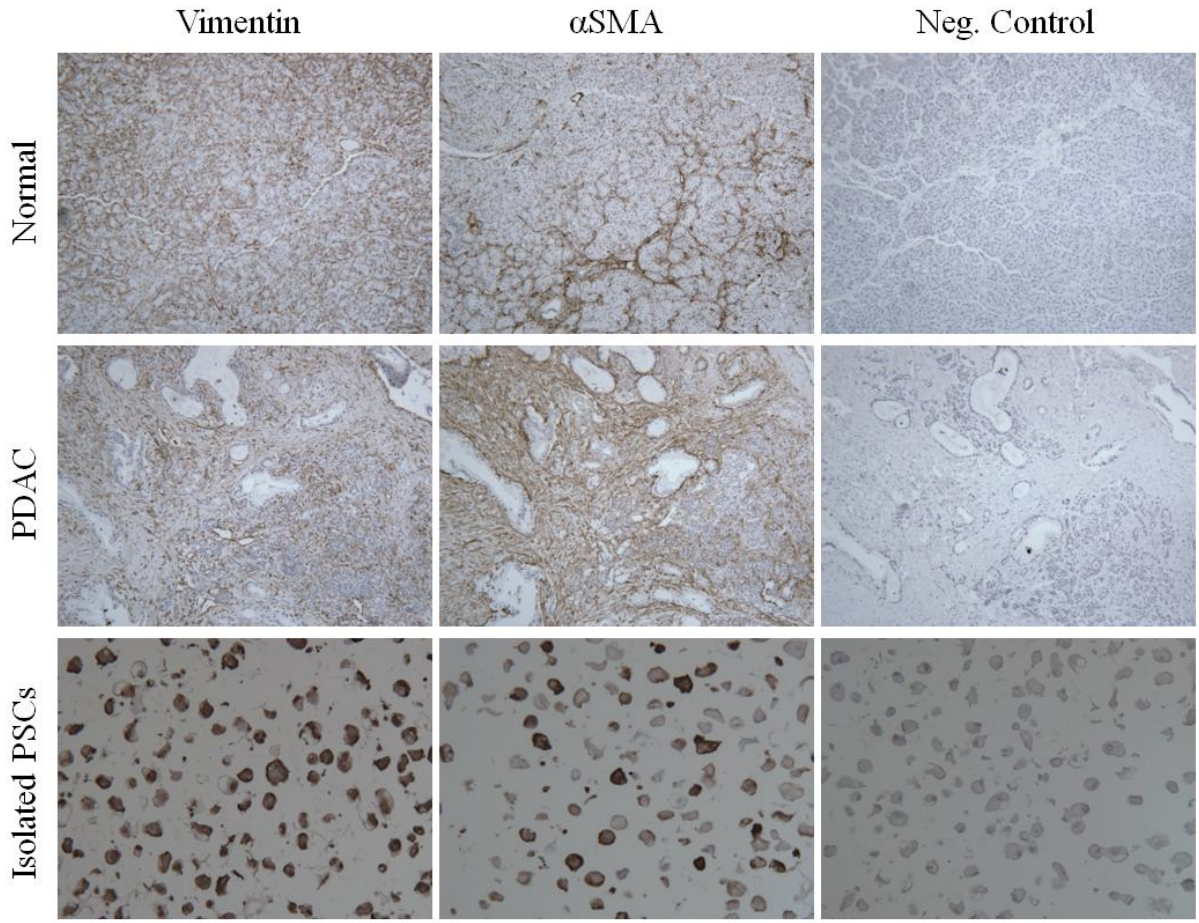
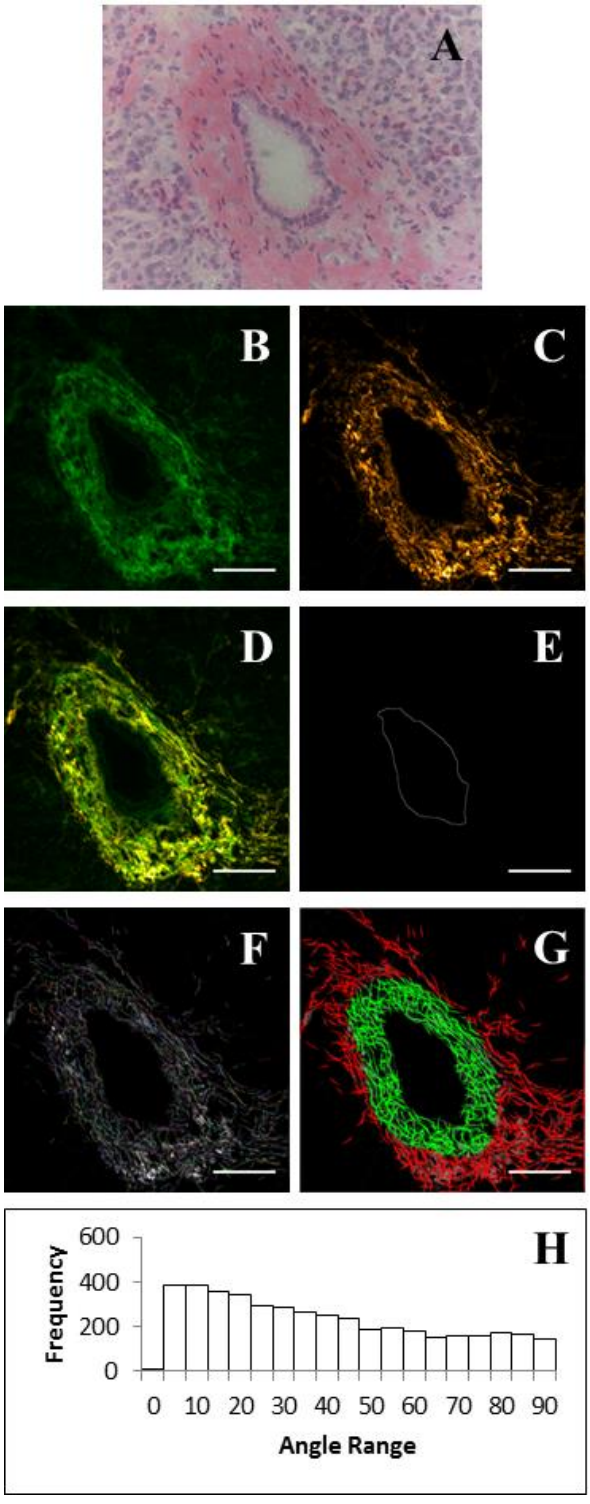


Fig. S1



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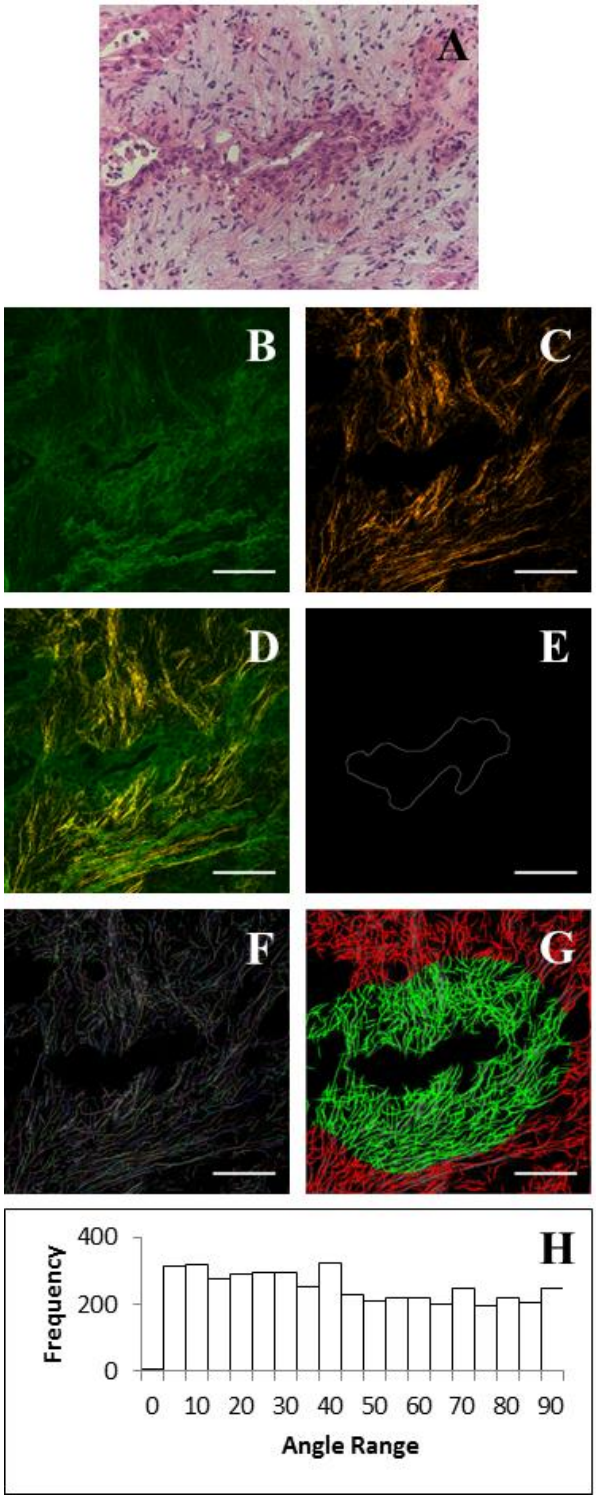


Fig. S3

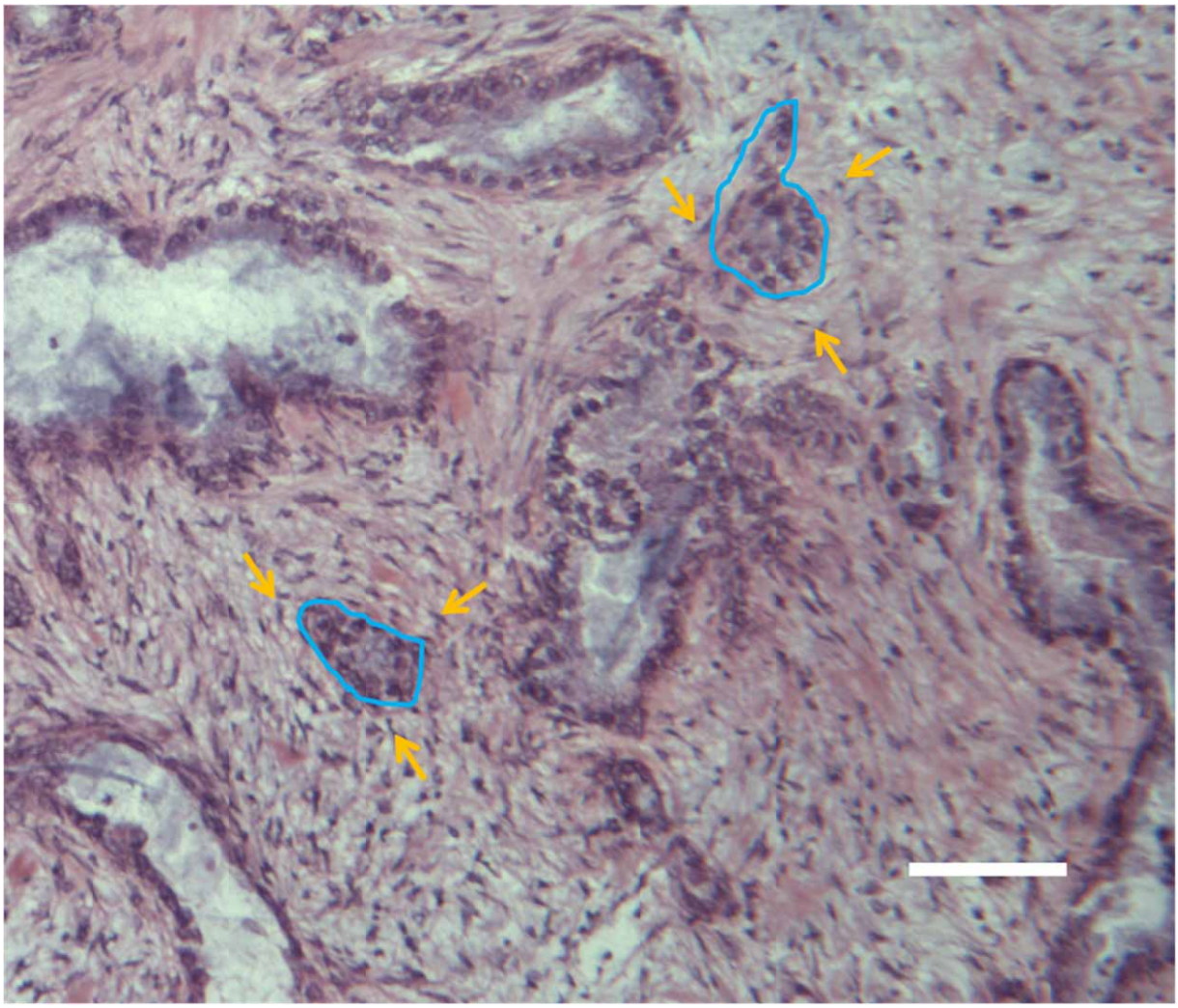
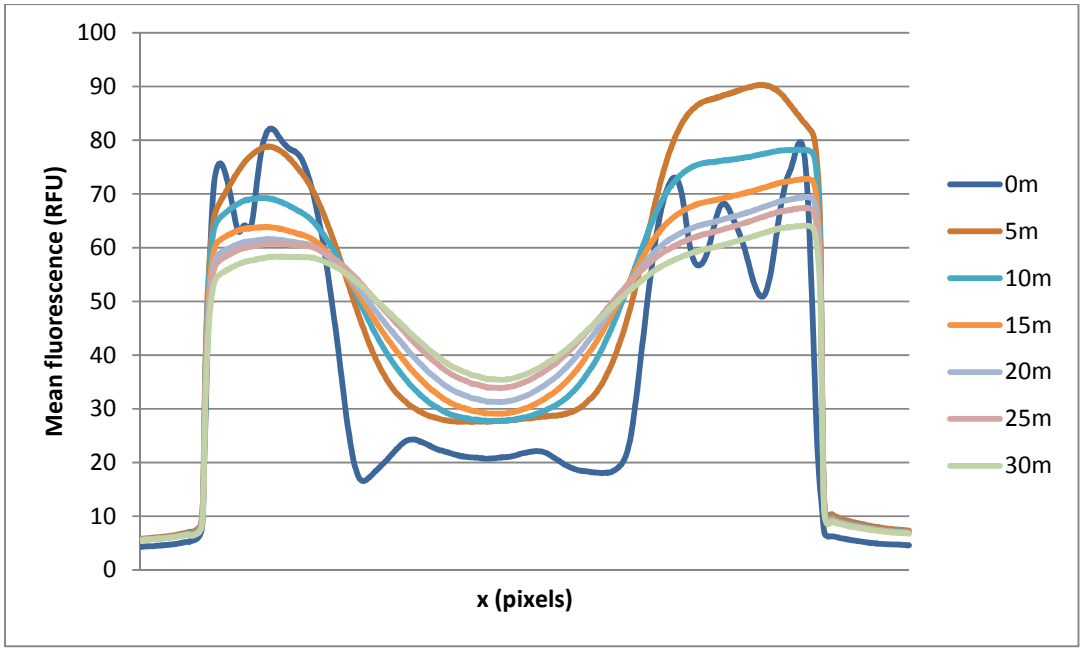
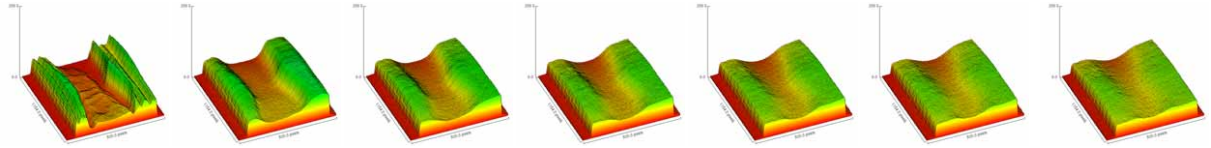


Fig. S4





B

Fig. S5

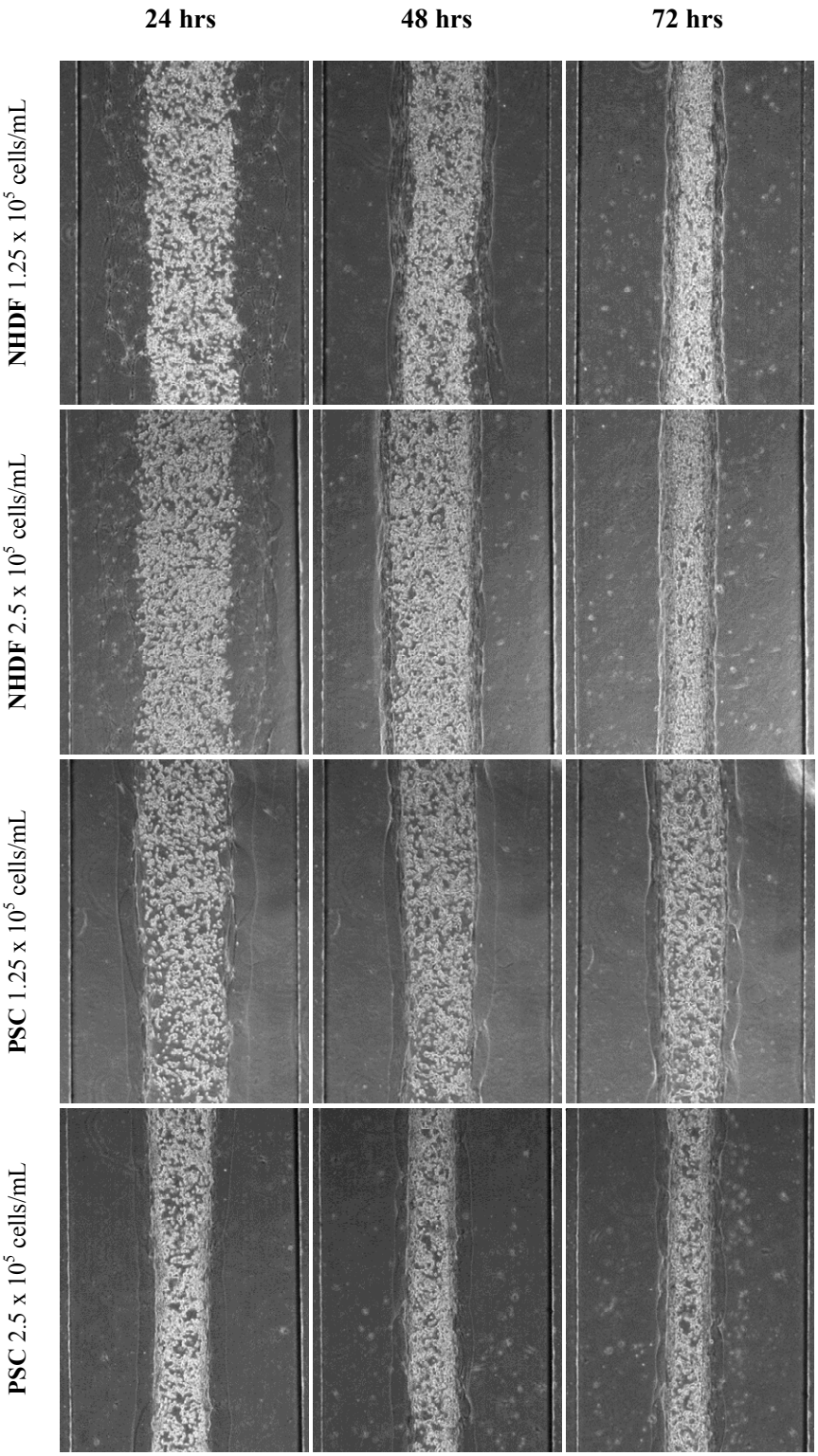


Fig. S6

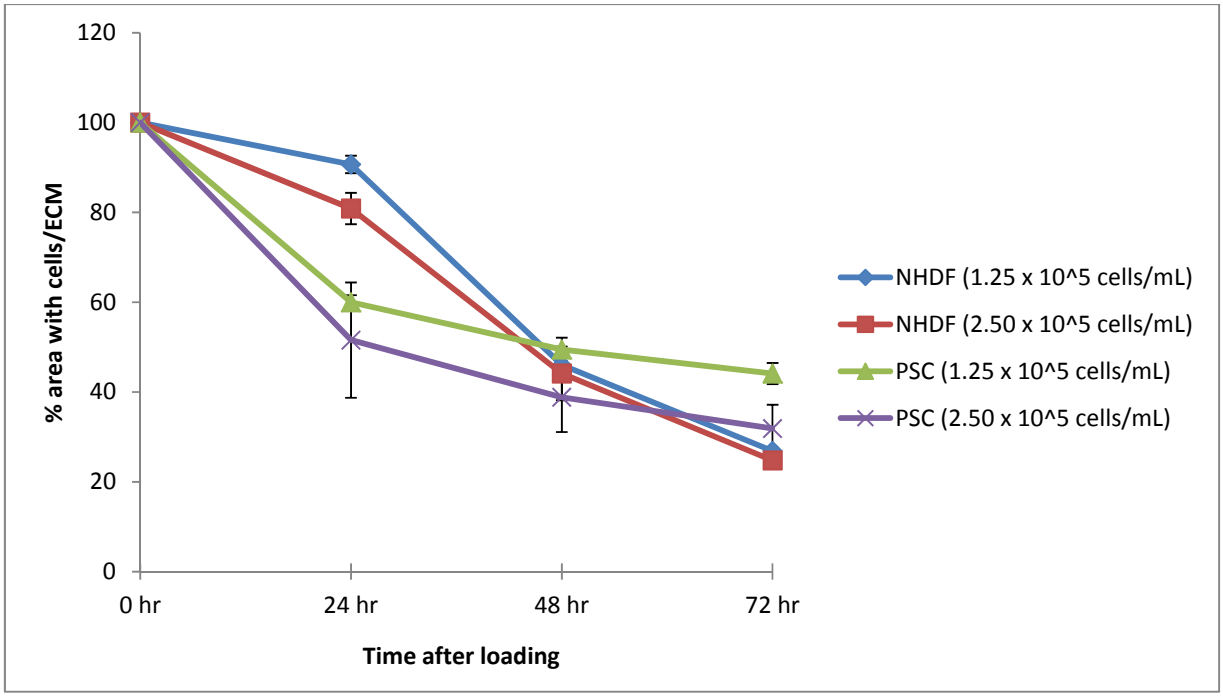


Fig. S7

Electronic Supplementary Material (ESI) for Lab on a Chip

Supplementary Movie Captions

Movie S1 Movie of SHG z-stack data obtained by optically sectioning through approximately 100 μm of frozen, intact human normal pancreas tissue.

Movie S2 Movie of SHG z-stack data obtained by optically sectioning through approximately 100 μm of frozen, intact human PDAC tissue.