3D reconstructed pancreas: A model capturing the unique tumor microenvironment and stromal architecture of pancreatic cancer

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Introduction

- The prognosis for people with pancreatic cancer is poor, with an average 5-year survival rate of 10% and only 3% for those with metastatic illness. Pancreatic cancer patients have few therapeutic options, and innovative therapies are sorely needed to improve treatment of the disease. This is mainly due to the late diagnosis and partially due to the biology of the disease.
- It has been shown that human pancreatic tumors have an outer layer of stiff extracellular matrix (ECM) that functions as a physical barrier, preventing drugs from penetrating the tumor (Piersma, 2020).[1]
- To address this with our model, we have incorporated a collagen capsule and have co-cultured pancreatic tumor cell lines with primary activated pancreatic stromal cells.
- We have created a 3D model of the pancreatic tumor microenvironment, the *Reconstructed Pancreas* (r-Pancreas), which is formulated to recapitulate the typical ECM of pancreatic tumors. We tested the response of BxPC-3, PANC-1 and Mia PaCa-2 pancreatic tumor cell lines embedded in this ECM to standard of care therapies. We demonstrated that gemcitabine was effective against pancreatic tumor cells cultured in r-Pancreas (IC_{50} = 0.4-0.8 µM), while 5-fluorouracil (5FU) was ineffective (IC_{50} not reached).
- In nude mice, subcutaneous PANC-1 tumors exhibited similar responses to gemcitabine and 5FU as demonstrated in the 3D model. Treatment with gemcitabine resulted in a Day 63 median ΔT/ΔC of 37% while 5FU resulted in a Day 63 median ΔT/ΔC of 102%.

Methods

- PANC-1 and MIA PaCa-2 were mixed with ECM and plated in a 96-well plate. For the 2D setup, ECM was not added and the cells were plated at the bottom of the wells. Pancreatic cancer-specific supplement was added into the media for both the r-Pancreas and 2D setup. Gemcitabine and 5FU were administered into the media 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured (Kirshner, 2022).[2]
- Collagen-1 (Corning; Durham, NC) was added into the transwell insert and allowed to polymerize. BxPC-3, PANC-1 and MIA PaCa-2 were mixed with ECM and plated in a 96-well plate. Pancreatic cancer-specific supplement was added into the medium. Gemcitabine was administered into the medium 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured accordingly.
- BxPC-3, PANC-1 and MIA PaCa-2, along with human pancreatic fibroblasts, were mixed with ECM and plated in a 96-well plate. Pancreatic cancer-specific supplement was added into the medium. Gemcitabine was administered into the medium 3 days after the cells had been plated. The Celltiter-Glo® 3D assay (Promega; Madison, WI) was performed at day 7 and the viability of the cells was measured.
- Female HSD: Athymic nude-*Foxn1^{nu}* mice were implanted with 1.0E+07 human PANC-1 cells (SC - high axilla) in 50% Matrigel. After staging, ten mice per group were treated with either vehicle, intraperitoneal gemcitabine at 100 mg/kg every 3 days for 4 cycles (IP, 100 mg/kg, Q3Dx4), or intraperitoneal 5FU at 100 mg/kg every 7 days for 3 cycles (IP, 100 mg/kg, Q7Dx3). Tumors were measured every 2 days from 18 to 81 days post-tumor implant.
- All animal work was performed in an AAALAC-accredited facility, in alignment with applicable animal welfare regulations and with predetermined humane euthanasia criteria on all studies.

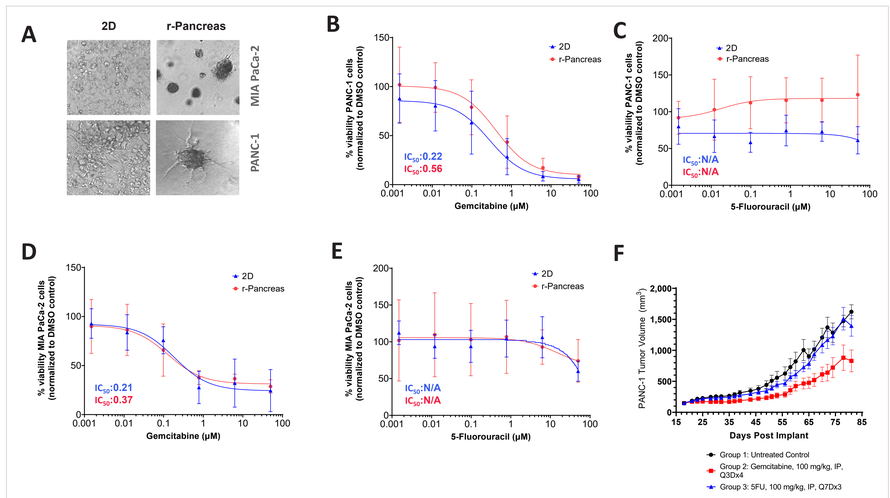
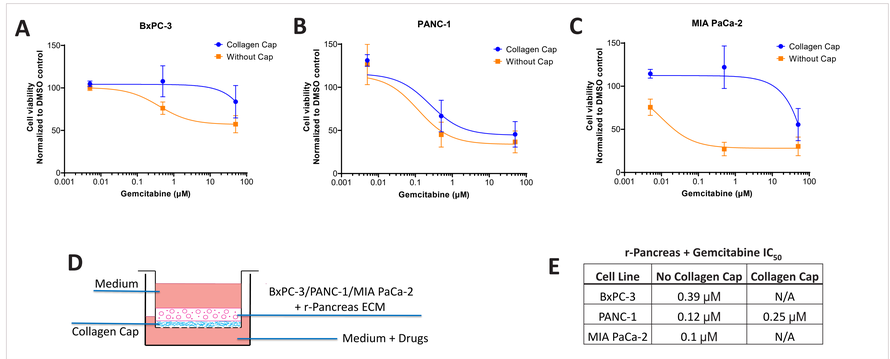


Figure 1. ***Reconstructed Pancreas*** **(r-Pancreas) ECM. A)** Brightfield images showing the tumor architecture comparison of 2D and r-Pancreas matrix. **B-C)** PANC-1 cytotoxicity assays with gemcitabine and 5FU in the 2D and r-Pancreas matrix. **D-E)** MIA PaCa-2 cytotoxicity assays with gemcitabine and 5FU in the 2D and r-Pancreas matrix. **F)** Mouse *in vivo* responses of subcutaneously implanted PANC-1 tumors to gemcitabine and 5FU.

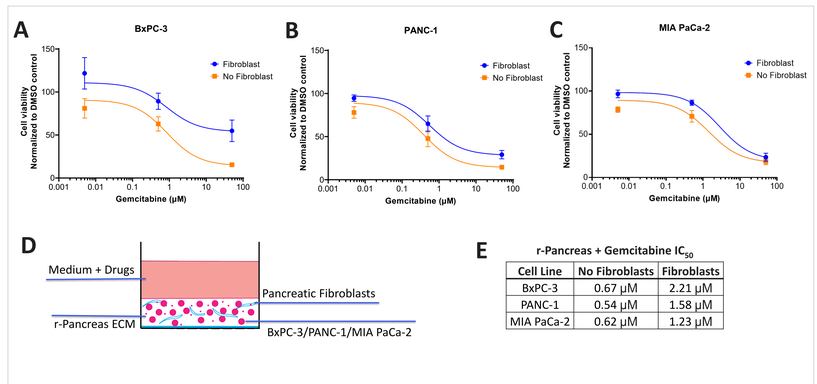


Cell Line	No Collagen Cap	Collagen Cap
BxPC-3	0.39 µM	N/A
PANC-1	0.12 µM	0.25 µM
MIA PaCa-2	0.1 µM	N/A

Figure 2. r-Pancreas ECM with a collagen cap. BxPC-3 **(A)**, PANC-1 **(B)** and MIA PaCa-2 **(C)** cytotoxicity assays with gemcitabine in the r-Pancreas matrix without and with a collagen cap. **D)** Diagram of the r-Pancreas matrix system with a collagen cap. **E)** IC_{50} values of the pancreatic tumor cell lines in the presence and absence of a collagen cap treated with gemcitabine.

References

1. Piersma, B., Hayward, M.K. and Weaver, V.M., 2020. Fibrosis and cancer: A strained relationship. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1873*(2), p.188356.
2. Kirshner, E.J., Ahlawat, A. and Ryou, M., 2022. Reconstructed Pancreas: A tumor-specific 3D culture platform for accurate evaluation of therapeutic agents in pancreatic cancer. *Cancer Research*, *82*(12_Supplement), pp.6030-6030.



Cell Line	No Fibroblasts	Fibroblasts
BxPC-3	0.67 µM	2.21 µM
PANC-1	0.54 µM	1.58 µM
MIA PaCa-2	0.62 µM	1.23 µM

Figure 3. r-Pancreas ECM with pancreatic fibroblasts. BxPC-3 **(A)**, PANC-1 **(B)** and MIA PaCa-2 **(C)** cytotoxicity assays with gemcitabine in the r-Pancreas matrix without and with pancreatic fibroblasts. **D)** Diagram of the r-Pancreas matrix system with pancreatic fibroblasts. **E)** IC_{50} values of the pancreatic tumor cells co-cultured without and with pancreatic fibroblasts treated with gemcitabine.

Results and Conclusions

- Capturing the components of the tumor microenvironment, which serve as both a physical barrier and a source of stromal-driven resistance to therapeutics, is one of the issues faced by drug developers searching for agents to combat pancreatic cancer.
- The addition of a collagen layer separating the r-Pancreas + BxPC-3, PANC-1 or MIA PaCa-2 mixture from the medium containing gemcitabine reduced the sensitivity of the cancer cells to gemcitabine mimicking the ECM around pancreatic tumors and preventing drug penetration. Incorporating a collagen-rich capsule increased physiological relevance of the r-Pancreas model.
- The addition of pancreatic fibroblasts to the r-Pancreas + BxPC-3, PANC-1 or MIA PaCa-2 mixture reduced the sensitivity of the cancer cells to gemcitabine demonstrating the protective effect of fibroblasts against drug treatment in pancreatic tumor models. The IC_{50} values for the pancreatic tumor cell lines treated with gemcitabine increased by two-fold when co-cultured with activated pancreatic fibroblasts, compared to control sets of tumor cells alone (IC_{50} = 1-3 µM). Creating a co-culture of primary activated pancreatic stromal cells and pancreatic tumor cell lines produced a more complete 3D model that permits the testing of potential therapeutic agents inside the pancreatic tumor microenvironment.
- The response of the human pancreatic cancer cell line, PANC-1, to gemcitabine and 5FU in 3D r-Pancreas ECM mirrored the *in vivo* response of subcutaneously implanted PANC-1 cells in female nude mice.
- Our data suggests, that to be clinically relevant, *in vitro* models of pancreatic cancer must integrate tumor-specific components of the microenvironment, including collagen cap and activated fibroblasts.

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