A 3D Microvessel Model of Pancreatic Cancer Revealing Tumor-Vascular Interactions and Intravasation

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Background

Pancreatic Ductal Adenocarcinoma

- A **highly lethal** and aggressive cancer
- Patients are often **diagnosed after metastasis occurs**
- Metastasis decreases patient survival
- Current **treatments** are largely **ineffective** [2]

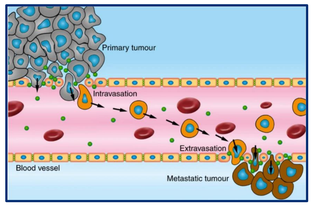


Figure 1. The metastatic cascade [4].

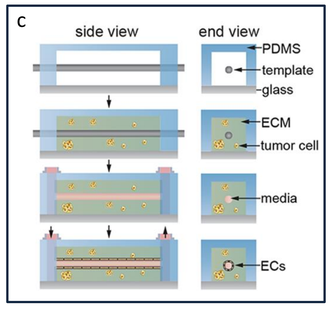
Insufficient Research Methods

- **Animal models** don't replicate the human tumor-vessel microenvironment
- **Cell lines** may undergo phenotypic or genetic alterations
- **2D *in vitro* cell cultures** don't replicate interactions between tumor cells and the extracellular matrix or gradients in pH, oxygen, and nutrients
- **3D *in vitro* co-cultures** of the tumor microenvironment don't demonstrate spatial and temporal dynamics of metastasis or response to luminal flow

Objectives

- Create a 3D *in vitro* model of the pancreatic tumor microenvironment to study cancer metastasis
- Design a ***process*** to collect perfusate and image intravasated cells
- Design a ***process*** to quantify characteristics of intravasated cells
- Design a ***process*** to quantify tumor-vessel interactions

3D Microvessel Model



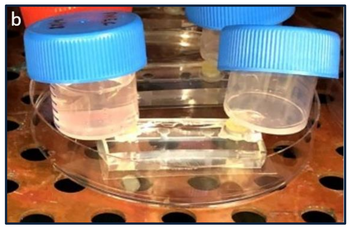


Figure 2. Model fabrication process. (a) Microfluidic device housing, addition of 7mg/ml collagen IV hydrogel embedded with hPDAC tumors, cylindrical template removal, endothelial cells seeded to form microvessel. (b) Two flow reservoirs placed upstream (left) and downstream (right).

Observations

1. Endothelial cells (VeraVecs™) and human pancreatic ductal adenocarcinoma cells (hPDACs) form **mosaic vessels**.
2. **Tumor cells intravasate** and flow downstream.

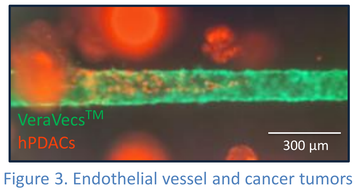
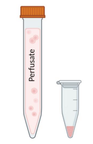


Figure 3. Endothelial vessel and cancer tumors revealing cancer cell intravasation.

Approximately 100 circulating tumor cells per mL were found in patients with pancreatic ductal adenocarcinoma (PDAC) [5].

Data Collection and Analysis Methods



Perfusate Collection and Imaging

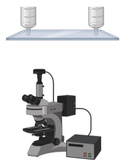
Immediately after seeding and every 24 hours thereafter

1. Transfer perfusate into tube
2. Concentrate perfusate objects via centrifuge
3. Disperse on hemacytometer
4. Image hemacytometer with epifluorescence microscopy

Microvessel Imaging

Immediately after seeding and every subsequent day

- Image microvessel with epifluorescence microscopy

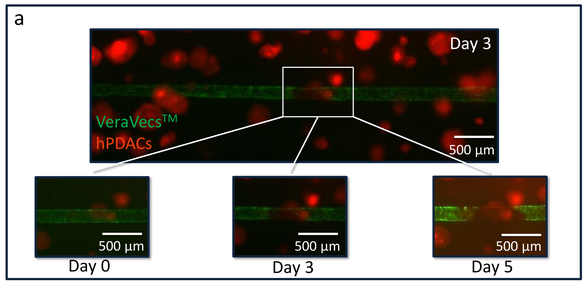


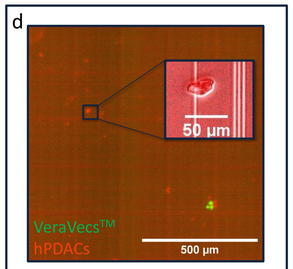
Analysis Methods: ImageJ

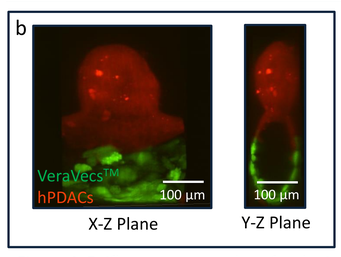
- Perfusate Images: Using fluorescent images, quantify number of objects and their areas by tracing perimeter
- Microvessel Images: Quantify number of tumor-vessel interactions

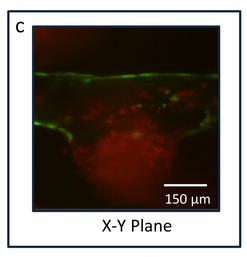
[1]

Intravasated Cells and Tumor-Vessel Interactions









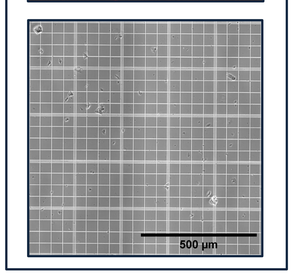


Figure 4. Epifluorescence and confocal microscopy images. (a) Fluorescent images of mosaic vessel formation over time. (b) Confocal images of a mosaic vessel. (c) Confocal image of a mosaic vessel shedding tumor cells into the microvessel lumen. (d) Concentrated perfusate dispersed on a hemacytometer revealing fluorescent hPDACs and VeraVecs™ (top) and the associated phase image (bottom).

Results

The area of a single hPDAC ranges from 130-415 µm² [3]. Therefore, **"objects"** refers to **tumor cells** and **tumor cell clusters**. Only data from objects with areas > 130 µm² are presented (n=4 biological replicates imaged over five days).

Table 1. Object area and cell cluster size comparison

Object area (µm2)	Cell cluster size
130-415	Single cells
416-999	3-7 cells
1,000-1,999	7-15 cells
2,000-9,999	15-75 cells
10,000+	75+ cells

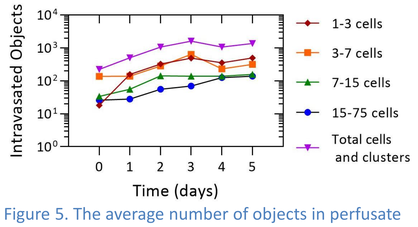


Figure 5. The average number of objects in perfusate within a specified area range as a function of time.

The intravasation of cancer cells and cancer cell clusters increases over time.

Results (Cont.)

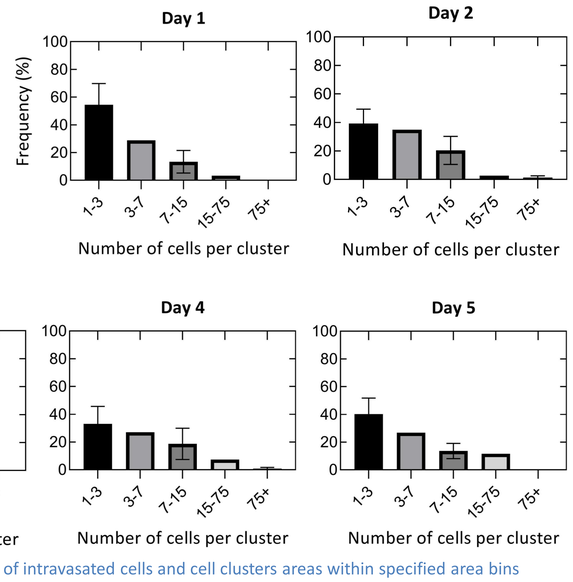
Days 1-3

1-7-cell clusters are most prevalent

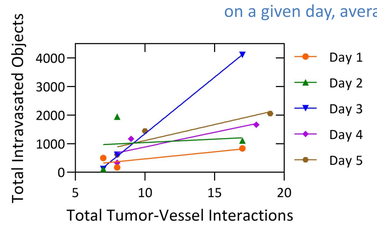
Days 4-5

7-75-cell clusters increase at the expense of single cells and smaller clusters

Intravasation of larger clusters becomes more prevalent with mosaic vessel growth.



Figures 6. The distribution of intravasated cells and cell clusters areas within specified area bins on a given day, averaged (with SEM) across samples.



The total number of intravasated cells and cell clusters is directly correlated with the number of tumor-vessel interactions.

Figure 7. Total intravasated objects as a function of tumor-vessel interactions. Each data point represents one sample on a given day. "Total Intravasated Objects" refers to total cell clusters of all sizes. "Total Tumor-Vessel Interactions" are defined as all points in which a tumor is within 50 µm of the microvessel in the x-y plane.

Conclusion

The *in vitro* 3D microvessel model successfully recapitulates tumor-vascular interactions and intravasation occurring during PDAC. The results suggest that at early timepoints, single cells and small clusters of cells intravasate at cell-cell junctions. At later timepoints, the results suggest that larger clusters of cells intravasate as a result of mosaic vessels. These results advance understanding of cancer intravasation mechanisms.

Future Directions

1. Explore the degree to which mosaic vessel formation contributes to intravasation
2. Develop a model for coupled intravasation and extravasation

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References

[1] Figures created with biorender.com
[2] Huang, W. et al., 2020, *Cancer Res.*
[3] Nguyen, A. V. et al., 2016, *Integr Biol.*
[4] Peng, F. et al., 2019, *Nat. Nanotechnol.*
[5] Poruk, K. et al., 2016, *Ann Surg.*