Modelling Juxtacrine Mediated Tumor-Fibroblast Interactions in Three-Dimensional Co-Cultures of Pancreatic Ductal Adenocarcinoma

Eric Struth
University of Massachusetts Boston

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MODELLING JUXTACRINE MEDIATED TUMOR-FIBROBLAST INTERACTIONS IN
THREE-DIMENSIONAL CO-CULTURES OF PANCREATIC DUCTAL
ADENOCARCINOMA

A Thesis Presented
by
ERIC STRUTH

Submitted to the Office of Graduate Studies,
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in partial fulfillment of the requirements for the degree of

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Applied Physics Program
MODELLING JUXTACRINE MEDIATED TUMOR-FIBROBLAST INTERACTIONS IN THREE-DIMENSIONAL CO-CULTURES OF PANCREATIC DUCTAL ADENOCARCINOMA

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ERIC STRUTH

Approved as to style and content by:

___________________________________
Jonathan Celli, Associate Professor
Chairperson of Committee

___________________________________
Joanna B Dahl, Assistant Professor
Member

___________________________________
Mohamed Amine Gharbi, Assistant Professor
Member

___________________________________
Jonathan Celli, Program Director
Applied Physics Program

___________________________________
Rahul Kulkarni, Chairperson
Physics Department
ABSTRACT

MODELLING JUXTACRINE MEDIATED TUMOR-FIBROBLAST INTERACTIONS IN THREE-DIMENSIONAL CO-CULTURES OF PANCREATIC DUCTAL ADENOCARCINOMA

May 2022

Eric Struth, A.S., Bunker Hill Community College
B.S., University of Massachusetts Boston
M.S., University of Massachusetts Boston

Directed by Professor Jonathan Celli

The tumor-microenvironment is a rich and complex milieu of mutated cancer cells and otherwise healthy cells engaged in dynamic interactions. Fibroblasts, the most abundant cellular component of human connective tissues, are implicated in a tumor promoting process known as stromal crosstalk. This stromal crosstalk is driven by numerous signaling pathways including contact mediated juxtacrine signaling and directed long distance paracrine signaling. Recent research suggests these signaling pathways are particularly important for two distinct types of fibroblasts. Mayofibroblastic cancer associated fibroblasts (MyCAFs) are associated with tumor suppression and shown to rely primarily on juxtacrine signaling, while inflammatory cancer associated fibroblasts (iCAFs) are associated with tumor
promotion and have been shown to rely on paracrine signaling in the tumor microenvironment. In this thesis two 3D co-culture platforms are utilized to model paracrine and juxtacrine signaling between pancreatic ductal adenocarcinoma (PDAC) and iCAF-like fibroblasts vs MyCAF-like fibroblasts. These studies implicate juxtacrine signaling as the primary mediator of in vitro tumor-fibroblast contractility. We further apply our juxtacrine model to differentiate fibroblast contractility in drug resistant vs. drug naïve PDAC co-cultures and observe drug resistant PDAC co-cultures exhibit a muted contractile response compared to drug naïve co-cultures. Finally, we attempt to use macroscopic tumor-fibroblast aggregates generated in juxtacrine experiments to study the effect of stromal depletion by photodynamic therapy on drug delivery. We demonstrate enhanced drug penetration in PDT pretreated PDAC spheroid homocultures, although stromal depletion experiments are ultimately inconclusive.
ACKNOWLEDGEMENTS

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# TABLE OF CONTENTS

**ACKNOWLEDGEMENTS** ........................................................................................................ vi

**LIST OF FIGURES** ........................................................................................................... ix

## CHAPTER

<table>
<thead>
<tr>
<th>1. INTRODUCTION</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 A brief introduction to cancer</td>
<td>1</td>
</tr>
<tr>
<td>1.2 Pancreatic ductal adenocarcinoma</td>
<td>2</td>
</tr>
<tr>
<td>1.3 Stromal crosstalk</td>
<td>3</td>
</tr>
<tr>
<td>1.4 Desmoplasia drives PDAC progression</td>
<td>5</td>
</tr>
<tr>
<td>1.5 Disrupting desmoplasia</td>
<td>6</td>
</tr>
<tr>
<td>1.6 Acquisition of chemoresistance</td>
<td>7</td>
</tr>
<tr>
<td>1.7 Photodynamic therapy</td>
<td>7</td>
</tr>
<tr>
<td>1.8 Modeling PDAC-stroma interactions in vitro</td>
<td>8</td>
</tr>
<tr>
<td>1.9 The adjacent overlay platform: a versatile 3D in vitro model for studying fibroblast contractility inn the tumor microenvironment</td>
<td>9</td>
</tr>
<tr>
<td>1.10 Applying the AOC model to differentiate juxtacrine mediated contractile interactions between PDAC spheroids and MyCAF-like vs. iCAF-like fibroblasts</td>
<td>10</td>
</tr>
<tr>
<td>1.11 mRNA sequencing assesses differential gene expression of drug naïve and drug resistant cell lines</td>
<td>11</td>
</tr>
<tr>
<td>1.12 Applying the AOC model to differentiate juxtacrine mediated contractile interactions in drug naïve and drug resistant tumor-fibroblast co-cultures</td>
<td>11</td>
</tr>
<tr>
<td>1.13 Evaluating an embedded overlay co-culture platform to test the effects of paracrine signaling in PDAC – fibroblast interactions</td>
<td>12</td>
</tr>
<tr>
<td>1.14 Using AOC 3D models to evaluate the effect of PDT treatment on drug delivery/penetration</td>
<td>12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>2. MATERIALS AND METHODS</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1 Cell culture</td>
<td>13</td>
</tr>
<tr>
<td>2.2 Adjacent overlay co-cultures</td>
<td>14</td>
</tr>
<tr>
<td>2.3 Embedded overlay co-cultures</td>
<td>16</td>
</tr>
<tr>
<td>2.4 RNA sequencing</td>
<td>18</td>
</tr>
<tr>
<td>2.5 Enrichment analysis</td>
<td>19</td>
</tr>
<tr>
<td>2.6 Microscopy</td>
<td>19</td>
</tr>
<tr>
<td>2.7 Bright field microscopy</td>
<td>20</td>
</tr>
<tr>
<td>2.8 Dark field microscopy</td>
<td>20</td>
</tr>
<tr>
<td>2.9 Phase contrast microscopy</td>
<td>20</td>
</tr>
<tr>
<td>2.10 Fluorescence microscopy</td>
<td>21</td>
</tr>
<tr>
<td>CHAPTER</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>2.11 Confocal microscopy</td>
<td>22</td>
</tr>
<tr>
<td>2.12 Image segmentation and spheroid distribution analysis</td>
<td>23</td>
</tr>
<tr>
<td>2.13 Velocimetric data generated with PIVlab</td>
<td>23</td>
</tr>
<tr>
<td>2.14 PDT, stromal depletion, and drug penetration</td>
<td>24</td>
</tr>
<tr>
<td>3. MEASUREMENT OF FIBROBLAST CONTRACTILITY IN PDAC AOC MODELS</td>
<td>26</td>
</tr>
<tr>
<td>3.1 Co-culturing PANC1 spheroids with stromal cells overlayed in the spheroid plane results in a contractile interaction and the formation of large heterogenous aggregates</td>
<td>26</td>
</tr>
<tr>
<td>3.2 PIV analysis provides a framework for analysis of fibroblast contractility utilizing the AOC model</td>
<td>31</td>
</tr>
<tr>
<td>3.3 Acquisition of drug resistance in the PANC1 cell line results in differential mRNA expression of genes which code for cell-adhesion</td>
<td>34</td>
</tr>
<tr>
<td>3.4 Acquisition of chemoresistance results in muted juxtacrine mediated fibroblast contractility in AOC experiments</td>
<td>35</td>
</tr>
<tr>
<td>4. MEASURING PARACRINE SIGNALING IN VITRO WITH AN EMBEDDED FIBROBLAST MODEL</td>
<td>39</td>
</tr>
<tr>
<td>4.1 The EOC method provides some insight into the role of paracrine signaling in the tumor microenvironment</td>
<td>39</td>
</tr>
<tr>
<td>5. PDT AND DRUG DELIVERY</td>
<td>41</td>
</tr>
<tr>
<td>5.1 Drug delivery is enhanced when paired with PDT pretreatment in 3D PANC1 spheroid homoculture</td>
<td>41</td>
</tr>
<tr>
<td>5.2 PDT pretreatment of AOC aggregates proves inconclusive</td>
<td>43</td>
</tr>
<tr>
<td>6. CONCLUSIONS AND FUTURE WORK</td>
<td>45</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>50</td>
</tr>
</tbody>
</table>
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Schematic representation of AOC co-culture model</td>
<td>15</td>
</tr>
<tr>
<td>2.</td>
<td>Schematic representation of EOC co-culture model</td>
<td>17</td>
</tr>
<tr>
<td>3.</td>
<td>Fluorescence microscopy image data from homoculture drug penetration experiments</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>10x phase contrast image of PDAC-fibroblast AOC</td>
<td>24</td>
</tr>
<tr>
<td>5.</td>
<td>Schematic representation of PDT setup</td>
<td>25</td>
</tr>
<tr>
<td>6.</td>
<td>AOC image data</td>
<td>27</td>
</tr>
<tr>
<td>7.</td>
<td>Differential AOC aggregation</td>
<td>28</td>
</tr>
<tr>
<td>8.</td>
<td>PANC1 homoculture and PANC1 AOC nodule size distributions (μm²) at 0 and 48 hrs</td>
<td>29</td>
</tr>
<tr>
<td>9.</td>
<td>Spatial nodule distribution for PANC1 homocultures and PANC1 AOCs at 0 and 48 hrs</td>
<td>30</td>
</tr>
<tr>
<td>10.</td>
<td>AOC average object speed over time</td>
<td>31</td>
</tr>
<tr>
<td>11.</td>
<td>PIVlab generated velocimetric data for PANC1 AOCs</td>
<td>32</td>
</tr>
<tr>
<td>12.</td>
<td>AOC acceleration</td>
<td>33</td>
</tr>
<tr>
<td>13.</td>
<td>Differential mRNA expression heatmap PANC1 vs PANC1-OR</td>
<td>34</td>
</tr>
<tr>
<td>14.</td>
<td>PANC1 and PANC1-OR AOC nodule size distribution (μm²) at t = 0 and 48 hrs</td>
<td>35</td>
</tr>
<tr>
<td>15.</td>
<td>2D representations of spatial distributions for PANC1 and PANC1-OR AOCs at 0 and 48 hrs</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td><strong>16.</strong></td>
<td>Differentiating fibroblast contractility in PANC1 vs PANC1-OR AOCs ........................................37</td>
<td></td>
</tr>
<tr>
<td><strong>17.</strong></td>
<td>EOC data .................................................................................................................................................40</td>
<td></td>
</tr>
<tr>
<td><strong>18.</strong></td>
<td>Homoculture drug penetration data .......................................................................................................42</td>
<td></td>
</tr>
<tr>
<td><strong>19.</strong></td>
<td>Plot of average DOX intensity versus nodule diameter ..........................................................................43</td>
<td></td>
</tr>
<tr>
<td><strong>20.</strong></td>
<td>Confocal image data of AOC aggregate nodules ....................................................................................44</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 A brief introduction to cancer

The term *cancer* is applied to myriad disparate diseases, each presenting unique symptoms and posing unique challenges. These diseases are underpinned by genetic mutation but owe as much of their existence to the versatility of the biological cell. Our everyday interaction with cells is almost universally with differentiated cells, having adopted their specialized phenotype to fulfill a particular purpose these cells seem as eternal as mountains, how could a skin cell be anything but a skin cell? But mountains erode, and organisms replenish cells continuously, and an organism’s entire genome is encoded in every cell. This same genetic code provides a plethora of latent possibilities waiting to be coopted by mutated cellular mechanisms. Random mutations occur and are passed on through reproduction, while most are innocuous, as mutations collect, they can impact the function of the cell. Certain increasingly well understood types of mutations create the necessary conditions for cancers to take root, and a common set of general traits shapes these diseases.\(^1,2\)
Hanahan dubs six such traits the Hallmarks of Cancer in the seminal work of the same title. Tumors proliferate unabated, hijacking the mechanisms that control cell population in order to multiply indefinitely. They also evade signals that suppress growth, avoiding active tumor suppression. Tumors resist the natural mechanisms of cell death such as apoptosis, and in some cases even use the carnage resulting from cell death for their own benefit. Cancer cells achieve replicative immortality, generating telomerase to replenish telomeres, the ever-shrinking protective ends of DNA strands. Tumors induce angiogenesis, the development of vasculature to deliver much needed oxygen and nutrition to the site of the tumor. And cancers metastasize, leaving the moorings of their origin tissues to invade throughout the host. Much progress has been made in the wider field of oncology with some cancers, such as breast cancer or prostate cancer, being exceptionally well characterized, and when caught in their earliest stages proven effective treatments render these diseases practically curable. For many cancers however, treatment and survivability remain elusive.

1.2 Pancreatic ductal adenocarcinoma
Pancreatic ductal adenocarcinoma (PDAC) is among the most lethal of human malignancies, claiming the lives of more than three quarters of those who are diagnosed at any stage within the first year. While many promising advances have been made in the lab, in practice most efforts to treat PDAC are ineffective at realizing prolonging survival. PDAC is an adenocarcinoma. Adenocarcinomas form in the glands that line the interior ducts of organs. PDAC tumor evolution can be broken into three key stages. The first stage is initiation, all
cancers begin with an initial somatic mutation. Adult pancreatic tissues proliferate slowly and in PDAC the initial mutation may occur decades before diagnosis. The next stage in PDAC evolution is clonal expansion, the original mutation must now proliferate and take root in pancreatic epithelial tissue. During clonal expansion, generations of cells proliferate and acquire additional mutations. Like a Darwinian nightmare, some of these additional mutations are advantageous for disease progression; synergizing with previous mutations, these adaptations lead to a stronger more evolved tumor. The final stage of PDAC evolution is invasion. Here the stroma which have enveloped and protected the tumor also play a key role in triggering invasion and establishing physical pathways for these invading tumor cells to leave the primary tumor nodule. Mediating these phases is collaboration between mutant cancer cells and otherwise healthy “normal” cells. This hijacking of healthy cells is driven by intercellular communication, and strongly implicates mutant cancer cells and normal fibroblasts as the key players in a process known as stromal crosstalk.

1.3 Stromal crosstalk

The constituent parts of an organ that aren’t specific to organ function, such as connective tissues and vasculature, are collectively known as *stroma*. The most common cellular component of stroma is the fibroblast. Fibroblasts are versatile cells occupying numerous roles in the body including the production of abundant collagen rich extracellular matrix (ECM), the scaffolding of the cellular microenvironment. Fibroblasts are also an essential participant in wound healing, being recruited to the site of a wound as part of the body’s
immune response where they generate inflammation.\textsuperscript{1,7} When fibroblasts are recruited by cancers, tumor and stroma engage in a complex chemical and physical dialogue.

Having been recruited via signaling pathways fibroblasts migrate to the site of the tumor and become cancer associated fibroblasts (CAFs).\textsuperscript{8–10} Cell-cell signaling between CAFs and cancer cells takes on numerous forms, but recent research has sought to better categorize signaling pathways. Recent studies have characterized two such phenotypic subtypes of CAFs, inflammatory cancer associated fibroblasts (iCAFs) and mayofibroblastic cancer associated fibroblasts (MyCAFs).\textsuperscript{11–13}

In the tumor microenvironment, iCAFs are located physically further from cancer cells and activate pathways that promote tumor proliferation and survival via paracrine signaling. Paracrine signaling is targeted intercellular signaling that involves chemical signals emitted by one cell that are then transmitted across distance in the cellular microenvironment where they are taken up by other specific cells activating a response.\textsuperscript{14} The role of paracrine signaling in mesenchymal stem cells, such as CAFs, has been reasonably well established. These interactions are dynamic, occur on the nanometer scales of molecule size, and are part of a complex milieux of chemical processes and signals.\textsuperscript{15,16} The challenges of scale and dynamic complexity make the specific paracrine signaling pathways in the tumor microenvironment favorable for study.
MyCAFs on the other hand, envelope tumors and form direct cell-cell adhesions, restraining tumor progression as a physical barrier to expansion while simultaneously insulating the tumor from traditional treatment delivery. Interactions between tumor cells and MyCAFs are directed by juxtacrine signaling. This juxtacrine signaling is fundamentally physical, induced by direct cell-cell adhesions between CAFs and cancer cells, and mediated by the genes that code for adhesion molecules in each cell type. Juxtacrine interactions, by definition, require contact between cells, and in principal, this should make it easier to experimentally observe juxtacrine signaling than it is to observe paracrine signaling.

1.4 Desmoplasia drives PDAC progression

Stromal crosstalk triggers a profound desmoplastic response, in which cancer cells recruit fibroblasts to the site of the tumor. This desmoplasia is a critical feature of the PDAC tumor microenvironment. Fibroblasts densely envelop the PDAC tumor and proliferate while simultaneously producing copious ECM as well as cancer promoting growth factors. The wall of fibroblasts physically impedes both the body’s natural defenses as well as the chemical attacks incurred by traditional cancer treatments, while secreted growth factors promote tumor development.

The physical manifestation of fibroblast proliferation in combination with excess ECM production is pressure exerted on the enveloped tumor. Newton’s 3rd law, and mantra of many a space age child, reminds us; for every action there is an equal and opposite reaction.
We know that pressure exerted on the tumor by the recruitment of stroma must be countered in turn by the tumor exerting pressure on the newly recruited stroma. This interplay of forces has wide-reaching implications for PDAC progression and efforts to treat it. A cruel irony of the recruitment of protective stroma, is that as fibroblasts proliferate and space becomes tight, cancer cells respond to this physical constraint by invading. The cancer cell sheds its epithelial phenotype, activating latent genes and restructuring the contents of its cytoplasm to develop into a spindly motile mesenchymal cell through a process known as epithelial to mesenchymal transition (EMT). The cells break free from the tumor nodule and invade into surrounding tissue where they move throughout the body until they can take root, revert to their epithelial state, and form new tumor nodules.23,24

1.5 Disrupting desmoplasia

Fibroblasts surround and protect PDAC tumors as well as promote their growth, so naturally one might assume disrupting stroma would assist in PDAC treatment.25,26 There is a growing body of evidence that supports the idea that targeting and depleting stroma might be a useful strategy for enhancing PDAC treatment.27,28 Notably, photodynamic therapy is implicated as promising method for eliminating stroma and enhancing drug delivery.29 Unfortunately, several studies counterintuitively find stromal depletion leads to more aggressive tumors and poorer survival, suggesting stroma may play a role in protecting the body from the tumor even as it simultaneously protects the tumor.30–32 While these mixed results may seem frustrating, they reveal a need to create new opportunities to more thoroughly explore
desmoplasia, stromal crosstalk and the roles of stroma in the PDAC tumor microenvironment in order to close these gaps in our understanding.

1.6 Acquisition of chemoresistance

Beyond physical barriers to drug treatment, PDAC cells which have acquired chemoresistance exhibit an invasive phenotype.\textsuperscript{33} These acquired invasive traits include increased EMT. Having undergone EMT, chemo-resistant PDAC have been shown to break off from a primary nodule and actively invade into model ECM in 3D in vitro studies.\textsuperscript{34} This could be related to a downregulation of genes which code for cell adhesion in chemo-naïve PDAC tumors. Differentiating expression of genetic information between chemo-naïve PDAC cells and chemo-resistant PDAC cells, as well as quantifying the physical interactions between such cells and the stroma of the tumor microenvironment could inform further methods for targeting more aggressive late-stage cancers.

1.7 Photodynamic therapy

A technique that has shown promise both in treating PDAC as well as for its potential use as a tool for disrupting stroma is photodynamic therapy (PDT).\textsuperscript{35-37} PDT is a photomedicine approach that harnesses light, oxygen, and photosensitive chemical agents to deliver precision targeted destruction of cancer cells while mitigating damage to surrounding tissues.\textsuperscript{38} The general approach to PDT involves two key phases. First, photosensitizer is
delivered to the treatment site. Once the photosensitizer has been delivered, the second phase requires the precision delivery of a specific range of wavelengths of visible light, tuned to activate the photosensitizer in the presence of oxygen.

Photodynamic therapy is a fundamentally quantum mechanical process. Energy from the treatment light dose excites electrons in the photosensitizer into a singlet-state, this excited state is highly unstable, and electrons quickly decay to lower energy levels. Many decay directly to their ground state emitting photons, via fluorescence, or generating heat. However, some excited molecules undergo intersystem crossing to a triplet state. This triplet state allows for the formation of reactive oxygen species, and this drives cellular toxicity in the target cancer cells.39

1.8 Modeling PDAC-stroma interactions in vitro

A handful of in-vitro 3D co-culturing methods for studying tumor-stroma interactions have been described and can be broken down into direct and indirect co-cultures.40 Direct co-cultures involve coculturing cancer cells and fibroblasts in the same vessel such that they can physically interact, while indirect co-cultures involve physically separating cancer cells from fibroblasts in such a way that they share the same media environment but are unable to physically interact.41 Most direct co-culturing methods involve monolayer cell culture. This 2D geometry fails to recapitulate the fundamentally 3D nature of real cancers and has mostly been limited to extracting chemical signaling information. While many indirect models
incorporate 3D cell culture, they can introduce artificial barriers to intercellular communication, and they often rely on unrealistic spatial distributions of constituent cells. In either case, cells grown on a rigid substrate like glass exhibit differential phenotypic expression than those grown on model ECM.

In this thesis, two platforms for studying in-vitro stromal interactions are described. One is an indirect embedded platform, where fibroblasts are embedded in model ECM and 3D tumor spheroids are grown on top of the ECM. The other is a direct co-culture adjacent platform, where fibroblasts are co-cultured alongside mature 3D tumor spheroids. Both models rely on interactions between tumor cells, fibroblasts, and a commercially available model ECM in order to more holistically recapitulate conditions and geometries observed in human PDAC. These platforms are then applied in an attempt to differentiate the role of juxtacrine vs paracrine signaling in PDAC desmoplastic response. The adjacent model is further applied to differentiating contractile interactions between chemo-resistant and chemo-naïve PDAC cells and stromal fibroblasts, and finally to a stromal depletion and drug penetration experiment.

1.9 The adjacent overlay platform: a versatile 3D in vitro model for studying fibroblast contractility in the tumor microenvironment

Contractile forces associated with PDAC desmoplasia are realized in a compelling fashion when stromal fibroblasts are introduced in vitro, upon a bed of Matrigel on which 3D PDAC tumor spheroids have been grown. Here we first characterize this in vitro model of the PDAC
desmoplastic reaction and then harness it to differentiate contractile interactions between cell lines in an adjacent overlay co-culture (AOC). Utilizing 3D direct co-culture methods to induce contractility, this novel test platform allows us to pry open some of the mystery of stromal cross talk, glean quantitative information about forces in fibroblast mediated contractile responses, and potentially examine strategies for stromal depletion and enhancing uptake of traditional treatments with targeted photomedicine.

1.10 Applying the AOC model to differentiate juxtacrine mediated contractile interactions between PDAC spheroids and MyCAF-like vs. iCAF-like fibroblasts

The AOC model described above is implemented to differentiate MyCAF and iCAF juxtacrine interactions with 3D PDAC spheroids. We begin by generating sequential image data via time lapse microscopy. Then, we demonstrate the use of a free open-source particle-image velocimetry analysis tool for analysis of cell co-migration and fibroblast contractility incurred by the addition of either MyCAFs or iCAFs to the co-culture. This allows for quantitative interrogation of biophysical interactions between tumor and stromal cells for each experimental condition. We find that while contractile juxtacrine interactions occur in both AOC models, desmoplastic forces are greater in the predominantly MyCAF fibroblast line.
1.11 mRNA sequencing assesses differential gene expression of drug naïve and drug resistant cell lines

mRNA sequencing is used to examine differential gene expression between PDAC cells (PANC1) and a previously generated stable oxaliplatin-resistant PANC1 subline (PANC1-OR). Results indicate acquisition of drug-resistance leads to a thoroughly altered landscape of mRNA expression. Among the many changes in mRNA expression, we find significant downregulation in genes that code for both cell-cell signaling and cell-cell adhesion. Downregulation of cell-cell signaling and adhesion genes could result in muted interactions between cancer cells and stromal fibroblasts due to fewer paracrine and juxtacrine signaling pathways.

1.12 Applying the AOC model to differentiate juxtacrine mediated contractile interactions in drug naïve and drug resistant tumor-fibroblast co-cultures

Having successfully implemented the AOC model to differentiate interactions between PANC1 and MyCAFs vs. iCAFs we then apply the AOC model to differentiate stromal interactions between MRC5 and PANC1 cells vs. oxaliplatin resistant PANC1-OR cells. Velocimetric measurements show a muted interaction between stroma and PANC1-OR cells compared to PANC1 cells. This is consistent with decreased mRNA expression of cell-signaling and cell-adhesion molecules as well as the acquisition of mesenchymal phenotypic traits characterized in the PANC1-OR subline.
1.13 Evaluating an embedded overlay co-culture platform to test the effects of paracrine signaling in PDAC – fibroblast interactions

Next, we evaluate an in vitro embedded-overlay co-culture (EOC) model. The model is applied to explore how 3D PDAC spheroids respond when indirectly cultured with fibroblasts embedded within a the Matrigel bed. Here, fibroblasts are embedded in Matrigel, and PANC1 cells are grown on top. After seven days the co-cultures are imaged. Image analysis reveals a differential landscape in the wells of each condition. This is best quantified with comparisons of spheroid size and analysis of spheroid circularity.

1.14 Using AOC 3D models to evaluate the effect of PDT treatment on drug delivery/penetration

Following the contractile event initiated by the addition of fibroblasts to the 3D co-cultures a complex macroscopic amalgamation of cancer cells and stroma is left in the well of the glass plate. These macroscopic aggregates potentially provide a platform for exploring stromal depletion strategies for enhancing drug delivery. Here experiments were performed using both 3D spheroid homocultures as well as the AOC platform to assess photodynamic therapy as a method for enhancing drug delivery and depleting stroma. In all experiments a PDT dose is delivered, followed by the administration of doxorubicin. While we demonstrate enhanced drug penetration following PDT in homoculture spheroids, AOC experimental results prove mostly inconclusive.
CHAPTER 2

MATERIALS AND METHODS

2.1 Cell culture

Immortalized PDAC cells (PANC1) and MyCAF-like human fibroblasts (MRC5) are acquired from ATCC and maintained in T75 cell culture flasks according to ATCC guidelines. DMEM and MEM (HyClone) are supplemented with 10% FBS (HyClone), 100IU/mL penicillin, 1% streptomycin (HyClone), and 0.5 mg/mL amphotericin B (Corning). Pancreatic Stellate Cells (PSC), an iCAF-like human fibroblast, are obtained from ScienCell. SteCM was supplemented with 2% FBS, 1% SteCGS, and 1% penicillin. PSC and MRC5 cells are used for experiments between the 3rd and 10th passages.

PANC1-OR is a stable oxaliplatin resistant sub-line of PANC1 developed and characterized by Gweyndolyn Cramer at the UMass Boston Cancer Biophysics Group. PANC1-OR cells are maintained in T75 flasks according to ATCC guidelines for the PANC1 parent line.
2.2 Adjacent overlay co-cultures

Multiple experiments make use of the AOC co-culture model. (Figure 1) In all cases, prior to plating cells, GFR Matrigel is thawed overnight at 4°C and kept on ice until use. Then, 225 ul of Matrigel is added to the center of each experimental well of a black-walled 24-well plate (Ibidi USA Inc.). After the addition of Matrigel the plate is agitated to ensure an even coat on the bottom of the well and then the plate is incubated at 37°C for 20 minutes allowing the Matrigel to solidify.

Initially we use the AOC model as a platform for evaluating juxtacrine interactions between PANC1 and MyCAF-like vs iCAF-like stromal fibroblasts. Here PANC1 cells are collected, and cell density is determined using an automated cell counter. Preparations of cells are made at a concentration of 7500 cells/mL of media. 1mL of the PANC1 cell preparation is plated in each of nine Matrigel coated wells of the prepared 24 well plate. The cells are allowed to incubate at 37°C and 5% CO₂ for 7 days. After 7 days of spheroid growth, MRC5 or PSC fibroblast cell lines are collected, counted, and a preparation of 1 x 10⁵ cells/mL of media is made. Media is removed from each well containing tumor spheroids. 1mL of the prepared MRC5-media solution is added to each of three wells of PANC1 spheroids. Then, 1mL of the prepared PSC-media solution is added to each of three additional wells of PANC1 spheroids. Finally normal cell-free media is added to the three remaining wells of PANC1 spheroids.
Figure 1: Schematic representation of AOC co-culture model. [A]: PDAC spheroids are grown for several days on a bed of Matrigel. Then, fibroblasts are introduced initiating a contractile reaction. The result is a large aggregation of fibroblasts and PDAC nodules. [B]: 10× Phase contrast experimental image of AOC aggregate. Scale bar = 200μm
We also apply the AOC model to differentiate contractile interactions between fibroblasts and drug naïve PANC1 and oxaliplatin resistant PANC1-OR cell lines. Matrigel is prepared as above, but here PANC1-OR cells are grown atop the Matrigel bed for seven days. After seven days a preparation of $1 \times 10^5$ MRC5 cells/mL of media is made. Media is removed from the wells containing spheroids and 1mL of the fibroblast preparation is introduced in each of three spheroid wells, while 1mL of cell-free media is added to the remaining wells.

2.3 Embedded overlay co-cultures

For the EOC model, MRC5 or PSC cells collected and suspended in Matrigel and 225ul of the fibroblast-Matrigel suspension is added to the center of each well. (Figure 2) After the addition of Matrigel the plate is agitated to ensure an even coat on the bottom of the well and then placed in the incubator until the Matrigel sets. PANC1 cells are collected, and cell density is determined using an automated cell counter. A preparation of PANC1 cells in media is made at a concentration of 7500 cells/mL of media. 1mL of the PANC1 cell-media preparation is plated in three MRC5-Matrigel$^\text{TM}$ suspension coated wells and three PSC-Matrigel suspension coated wells. The cells are allowed to incubate at 37°C and 5% CO$_2$ for 7 days before darkfield imaging is performed.
**Figure 2:** Schematic representation of EOC co-culture model. [A]: Fibroblasts are embedded in Matrigel and PDAC spheroids are grown on top. [B]: 10x Phase contrast experimental image of EOC spheroids. Scale bar = 200μm
2.4 RNA sequencing

The quality of the raw fastq files were assessed using FastQC (v.0.11.5). Adaptor sequences, “AGATCGGAAGAGCACACGTCTGAACTCCAGTCA”, and “AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT” were trimmed from the 3’ end of the reads using Cutadapt form the Trime Galore package (v.0.4.2). The trimmed reads were mapped against the human reference genome (Ensemble, GRCh38) using STAR/2.5 using default parameters. To map the miRNA reads, annotations from http://www.mirbase.org/ftp.shtml were used to index the genome. The average alignment rate was 96%. The sorted SAM files generated by STAR were used to estimate the transcript abundance per sample using featureCount from the Subread package (v.1.6.2). Gene expression analysis was performed using the edgR Bioconductor R package (v.3.24.3). The edgeR TMM method (trimmed mean of M values) was applied to the filtered genes utilizing the DGElist(), calcNormFactors(), estimateGLMCommon Disp(), estimateGLMTrendedDisp(), estimateGLMTagwiseDisp() functions. The glmFit and glmLRT functions from edgeR were used to fit a negative binomial generalized log-linear model to the read counts. The expression of the genes was ranked by logFoldChange (logFC) and false discovery rate (FDR). Differentially expressed genes (DEGs) were determined using abs(logFC) > 2 and FDR < 0.01 cutoffs, resulting in 1342 protein coding DEGs.
2.5 Enrichment analysis

The DEGs were utilized for enrichment analysis. The gene ontology (GO) was performed using the g:Profiler with g:SCS (https://biit.cs.ut.ee/gprofiler). GO terms and KEGG and Reactom pathways with significant overlap with up and down regulated genes were determined (FDR < 0.05) and visualized with Heatmaps using the ComplexHeatmap R package (v.1.20.0). To run the gene set enrichment analysis (GSEA), we used the fgsea Biocondoctor R package (v.1.9.5). The top pathways (P-value < 0.05) ranked by normalized enrichment scores (NES) were plotted in the visualization using the plotGseaTable function from fgsea R package with gseaParam set to 0.5.

2.6 Microscopy

Image data was acquired using two instruments. Darkfield, fluorescence, and confocal imaging utilized a Zeiss Axio Observer.Z1 microscope produced by Carl Zeiss Microscopy. This Zeiss microscope is a powerhouse imaging platform with wide ranging versatility. Time-lapse phase contrast and bright field imaging is performed with a LifeSciences EVOSfl Microscope. The family sedan to the Zeiss’s luxury coupe, what the EVOS lacks in resolving power and overall tunability it makes up for in utilitarian reliability. This includes the availability of a stage mounted incubation chamber facilitating the automated acquisition of live cell time-lapse imaging over multi-day experiments.
2.7 Bright field microscopy

Bright field microscopy is the most basic of microscopy techniques. Light is incident upon a sample. The sample absorbs some of this light creating contrast. This leads to a relatively dark sample on a light background. Bright field microscopy is simple and relatively easy to implement but can lead to difficulties resolving very transparent materials such as many biological tissues. Here time-lapse bright field images are captured with a 1.25x objective on the EVOSfl microscope. Images are collected in ten-minute increments over the course of four days.

2.8 Dark field microscopy

Dark field microscopy results in a bright sample on a dark background. This is achieved by blocking light from directly entering the objective lens. An opaque disk is placed between the light source and a condenser lens before the specimen. This results in a cone of light reaching the sample. The trajectory of the cone of light rays is such that only light reflected off the specimen reaches the viewer. Dark field images presented here are collected with the Axio Observer.Z1 utilizing a 5x objective.

2.9 Phase contrast microscopy

Like dark field microscopy phase contrast microscopy results in a relatively bright object on a relatively dark background. The key difference is that in the dark field method light is
physically blocked between the source and the specimen, resulting in a cone of light-rays incident on the specimen. In phase contrast microscopy the trajectories of light-rays are altered after reaching the specimen in order to enhance contrast. Here time-lapse phase contrast images are obtained with the 10x objective of the EVOSfl microscope. Images are collected in ten-minute increments over the course of four days.

2.10 Fluorescence microscopy

Fluorescence microscopy relies on both the delivery of a fluorescent dye, or fluorophore, to a specimen, as well as quantum mechanics. Light of specific wavelength incident on a fluorophore delivered to a specimen leads to excitation of electrons in the fluorophore to a higher energy state. As some of these electrons return to their stable energy state, they each emit a photon of light with a specific wavelength. Using optical filters, we can select that specific wavelength to resolve only light emitted by the fluorophore of interest. Here fluorescence imaging is obtained utilizing the Axio Observer.Z1 microscope. (Figure 3)
**Figure 3:** Fluorescence microscopy image data from homoculture drug penetration experiments. Live cells are stained with Calcein (green) and doxorubicin fluorescence is colored red. [left]: PANC1 spheroids treated only with doxorubicin. [right], PANC1 spheroids pretreated with BPD-PDT followed by doxorubicin. Dox channel intensity is measured and compared for each condition. Scale bars = 100um

### 2.11 Confocal microscopy

Confocal microscopy is an advanced imaging technique capable of incredibly high-resolution imaging of specimens. With confocal imaging utilized here, a highly focused laser beam scans a specimen at preselected depths. Each pass creates an optical section at a set depth, and those sections can be stitched together into a 3D representation of the specimen. The tunability of the laser source, and the filtering capabilities also allow for very specific excitation of fluorophores, and imaging of resulting fluorescence emissions. Here confocal microscopy is applied to drug penetration studies using the Axio Observer.Z1 microscope platform, with a 40x objective.
2.12 Image segmentation and spheroid distribution analysis

For all experiments image data is segmented with Matlab image processing tools. Properties regarding object size and shape are extracted from segmented images. Segmented image data from AOC experiments is utilized to produce spheroid size histograms, as well as 2D spheroid special distribution histograms. Segmented data from the EOC experiment is utilized to compare object size and circularity in EOCs to PANC1 homocultures.

2.13 Velocimetric data generated with PIVlab

Particle image velocimetry (PIV) was developed in the nineteen eighties and has been applied extensively to understand how solid objects flow in fluids. A common technique for micron scale PIV experiments involves suspending fluorescent, or otherwise distinguishable, nanoparticles in a fluid to measure how particles move in dynamic systems. Methods like these require expensive, specialized materials, and are not necessarily practical for measuring dynamic biological systems. More recently, powerful, free, readily available computational methods for performing PIV analysis on sequences of image data have become available.

Here velocimetric data is obtained using PIVlab v1.43 in Matlab. For 10x image data pre-processing is done in PIVlab. CLAHE, high pass, intensity capping, and denoise filters are all enabled in order to optimize in-app segmentation for analysis. For 1.25x bright field image data, image data is first segmented, and preprocessing is disabled in PIVlab. In both cases an 800x800 pixel region of interest is chosen to eliminate optical anomalies at the edges of the
image field. Multi-pass PIV analysis is completed utilizing the fast Fourier transform (FFT) window deformation algorithm generating velocimetric data. (Figure 4) Object speed is then averaged across each frame providing a basis for comparing the relative changes in kinetic energy under each experimental condition.

**Figure 4:** 10x phase contrast image of PDAC-fibroblast AOC. [A]: Frame coincides with peak object speed during in-progress nodule aggregation. Scale bar = 200um [B]: Segmented version of same image with overlayed PIVlab generated velocity vector map. Scale bar = 200um

2.14 PDT, stromal depletion, and drug penetration

Evaluation of PDT related enhancement of drug penetration is done using the AOC model described above, as well as PANC1 spheroids in homoculture. In the case of PANC1 3D homoculture experiments, PANC1 is plated and grown for 10 days. For AOC experiments, PANC1 is grown for 7 days, MRC5 cells are introduced as described in the AOC model above, and aggregates are allowed to coalesce for three days. At this point the photosensitizer
BPD is added to three wells of each experimental condition and allowed to accumulate. Then, light doses of 10J are delivered to each of the three wells for each condition utilizing the PDT dosimetry apparatus. (Figure 5) The following day 50 microgram doses of doxorubicin, an established chemotherapy agent that happens to have fluorescent properties, are delivered to all experimental wells. Fluorescence and confocal images are taken and analyzed.

Figure 5: Schematic representation of PDT setup. A diode laser with adjustable temperature and current serves as the light source. The laser is incident on the specimen stage, and a ThorLabs shutter is placed in the light-path to allow for computer-controlled dosimetry.
3.1 Co-culturing PANC1 spheroids with stromal cells overlaid in the spheroid plane results in a contractile interaction and the formation of large heterogenous aggregates

We performed several AOC experiments evaluating interactions between PANC1 tumor spheroids and either MRC5 or PSC fibroblastic cells. It has been shown that intercellular interactions between tumor cells and stroma are mediated by a combination of paracrine and juxtacrine signaling. When fibroblasts are introduced on top of a Matrigel bed upon which PANC1 spheroids have been grown, profound changes occur. Fibroblasts unfurl, each extending protrusions outward until they encounter a tumor nodule and forming a mesh-like network of fibroblasts and spheroids across the ECM surface. Having made contact with spheroids, this network of fibroblasts begins to contract, slowly pulling nodules into macroscopic aggregates of tumor cells and stroma. These aggregate nodules effectively recapitulate the dense networks of fibroblasts interspersed with tightly packed tumor nodules observed in human cancer in the wells of a plate. (Figure 6)
Figure 6: AOC image data. First row: 1.25x brightfield time-lapse images of fibroblast contraction in an AOC at t = 0, 24, and 48 hours. Scale bars = 1600um. Rows two and three: 10x phase contrast images of fibroblast contraction at t = 0, 3, 6, and 9 hours. Local contractile events occur during the initial acceleratory phase, while major contractile aggregation takes place over much longer time scales. Scale bars = 200um
Image data qualitatively differentiates AOCs from PDAC spheroid homocultures. (Figure 7) PANC1 are co-cultured with predominantly MyCAF-like MRC5 fibroblasts, profound aggregation is observed, but in PSC-AOCs a muted aggregation occurs, apparent but less dramatic.

**Figure 7:** Differential AOC aggregation. 10x Phase Contrast time-lapse images of PANC1-MRC5 and PANC1-PSC AOCs and homoculture spheroids at T = 0, 24 and 48 hrs. MRC5 and PSC AOCs both demonstrate clear aggregation, while PANC1 homoculture spheroids show little signs of movement. Scale bars = 200μm
Nodule size distribution histograms suggest mixed results. (Figure 8) MRC5 AOCs demonstrate a dramatic shift in nodule size between hour zero, the introduction of fibroblasts, and the 48-hour mark. (Figure 8, [Center]) PSC AOCs and PANC1 spheroids grown in homoculture show some shift in size towards slightly larger objects, an expected result of normal proliferation, but the spheroids remain smaller and more abundant in both compared to the MRC5 AOC aggregates. (Figure 8, [Right and Left])

![Nodule size distribution histograms](image)

**Figure 8:** PANC1 homoculture and PANC1 AOC nodule size distributions ($\mu$m$^2$) at 0 and 48 hrs.

Analyzing centroids of segmented images, we’re able to render 2D spatial distributions of nodules atop Matrigel. (Figure 9) After 48 hours both PANC1 AOC experiments present an environment with fewer and larger objects and with large swaths of empty Matrigel compared to PDAC spheroid homocultures. This is illustrated dramatically in the MRC5
AOCs where, after 48 hours, wells are dominated by a few very large co-mingled nodules (Figure 9, [Center]). While less dramatic than MRC5 AOCs, PSC AOC distributions also show greater aggregation when compared to PANC1 spheroids in homoculture. (Figure 9, [Right and Left]). PANC1 homocultures have no fibroblasts with which to directly interact, tumor spheroids remain largely dispersed across the Matrigel, in fact changes in spatial distribution are impacted more by nodules growing into each other through normal proliferation than by nodules migrating across the well. In contrast, in the AOC experiments PANC1 are free to physically interact with fibroblasts. Here, the fact that AOCs cultured with MyCAF-like MRC5 aggregate significantly more when compared to AOCs cultured with iCAF-like PSCs demonstrates that AOC fibroblast contractility is mediated by juxtacrine interactions.

**Figure 9:** Spatial nodule distribution for PANC1 homocultures and PANC1 AOCs at 0 and 48 hrs.
3.2 PIV analysis provides a framework for analysis of fibroblast contractility utilizing the AOC model

Time-lapse image data is processed in Matlab with the open source PIVlab tool. When fibroblasts are introduced in the same plane as PANC1 nodules atop the Matrigel bed, the resultant physical interaction occurs in two distinctive regimes, an initial acceleratory regime, followed by a gradual return to equilibrium. We note that the initial acceleratory regime appears linear and is driven by the early aggregation of smaller nodules, while the deceleration regime is nonlinear as larger intermediate aggregates settle into their final conglomerate. The linearity of the acceleration regime suggests a limit on juxtacrine mediate tumor fibroblast contractile forces. [Figure 10]

![Figure 10: AOC average object speed over time. From [A] to [B] we see a linear acceleratory regime dominated by local contractile aggregation events, within the next ten hours [C] aggregates have largely reached their final shape [D]. Scale bars = 200um](image-url)
Velocimetric data from PIVlab highlights characteristic differences between PANC1 AOCs and PANC1 homocultures. (Figure 11) PANC1 nodules grown in homoculture exhibit little change in particle speed over time, with a constant average background speed. While MRC5 and PSC AOCs both exhibit the characteristic dual velocimetric regime (Figure 11, [A]). Average AOC speed over the duration further highlights the differential responses of the three experimental conditions, with MRC5 AOCs maintaining the highest overall mean object speed. (Figure 11, [B])

**Figure 11:** PIVlab generated velocimetric data for PANC1 AOCs. [A]: MRC5 (purple) PSC (blue), and PANC1 3D homocultures (red). MRC5 and PSC AOCs both demonstrate the characteristic dual regime contractile response when fibroblasts are introduced, while homocultures maintain relatively constant background dynamics. [B]: Analyzing average speed over the duration of the experiment we find MRC5 AOCs (purple) have greatest overall average speed with PSC AOCs (blue) also demonstrating increased activity relative to PANC1 homocultures (red). It is evident that iCAF-like PSCs demonstrate a muted response compared to the MyCAF-like MRC5 fibroblasts in AOCs.
Velocimetric data further differentiates interactions based on stromal cell lines. MyCAF like MRC5 indicate a strong, uniform acceleratory phase where as iCAF like PSCs exhibit a muted acceleratory phase when compared with MRC5 AOCs. (Figure 12, [A]) MRC5 AOC acceleration is nearly twice that of PSC AOC acceleration. (Figure 12, [B])

**Figure 12:** AOC acceleration. [A]: Looking at the acceleratory regime we find differential contractile acceleration between each MRC5 AOCs (purple) and PSC AOCs (blue). [B] Average acceleration in MyCAF-life MRC5 mediated contractile events is twice that of those mediated by iCAF-like PSCs in AOCs.
3.3 Acquisition of drug resistance in the PANC1 cell line results in differential mRNA expression of genes which code for cell-adhesion

PANC1-OR cells, which have acquired drug resistance, express a more invasive phenotype marked in part by acquisition of mesenchymal physical characteristics. Using mRNA sequencing, we found depleted expression of genes which code for cell adhesion molecules in PANC1-OR. (Figure 13)

![Gene expression of Cell Adhesion Genes](image)

**Figure 13:** Differential mRNA expression heatmap PANC1 vs PANC1-OR.
3.4 Acquisition of chemoresistance results in muted juxtacrine mediated fibroblast contractility in AOC experiments

When grown on Matrigel drug resistant PANC1-OR cells still form nodules, but when MRC5 cells are introduced, there is a differential response when compared with drug-naïve PANC1 cells. Here, histograms of nodule size show marked differentiation between PANC1 AOC aggregation vs PANC1-OR AOC aggregation. (Figure 14)

![Histograms of nodule size distribution (μm²) at t = 0 and 48 hrs.](image)

**Figure 14:** PANC1 and PANC1-OR AOC nodule size distribution (μm²) at t = 0 and 48 hrs. In both cases we see a shift toward larger nodules, a sign of aggregation, but this shift is more pronounced in the PANC1 AOCs than in PANC1-OR AOCs.
In each case analysis of nodule spatial distributions also strongly suggests nodule aggregation. Significant portions of the field of view are centroid free in both AOC conditions after 48 hours. (Figure 15)

**Figure 15:** 2D representations of spatial distributions for PANC1 and PANC1-OR AOCs at 0 and 48 hrs. In both cases image fields are dominated empty Matrigel with fewer objects spaced further apart after 48 hours.
Analyzing velocimetric data we again see dual acceleratory and relaxation regimes. (Figure 16 [A]) Like the PANC1-PSC AOCs, the acceleratory regime resulting from PANC1OR-MRC5 AOCs are muted when compared with the PANC1-MRC5 AOCs, which is consistent with cells exhibiting reduced expression of genes which code for cell-cell adhesion as they are unable to form the physical bonds necessary to facilitate the juxtacrine response. (Figure 16 [C] & [D])

**Figure 16:** Differentiating fibroblast contractility in PANC1 vs PANC1-OR AOCs. Observing PANC1 (purple) and PANC1-OR (green) velocimetric data, we again see the characteristic dual regime contractile event in each case however we see the overall increase in activity is sustained deeper into the relaxation regime in PANC1-OR AOCs. [B]: The overall average speed for PANC1-OR AOCs is greater than the overall average speed in PANC1 AOCs. PANC1-OR homoculture object speed (dark blue) is also greater than PANC1 homoculture object speed (red). [C] & [D]: PANC1 AOCs demonstrate greater acceleration than PANC1-OR AOCs. mRNA data suggests downregulation of cell-adhesion genes in PANC1-OR which could account for the muted contractile response.
In this case the relaxation regime also indicates marked differences between AOCs. PANC1OR AOCs show a much shallower and noisier relaxation profile, never approaching pre-co-culture velocimetric conditions over the duration of data collection. Data also suggests homoculture PANC1OR spheroid cell cultures are more dynamic than drug-naïve cell-cultures with greater overall average speed, but this differential response doesn’t in and of itself account for the differences observed in the relaxation periods of AOCs.
CHAPTER 4

MEASURING PARACRINE SIGNALING IN VITRO WITH AN EMBEDDED FIBROBLAST MODEL

4.1 The EOC method provides some insight into the role of paracrine signaling in the tumor microenvironment

Coculturing PANC1 spheroid atop a Matrigel bed with embedded fibroblasts results in a subjectively similar in vitro landscape. (Figure 17 [A], [B], [C]). Experimental image data shows nodules distributed across the Matrigel bed with limited differentiation between experimental conditions. Digging into the image data we note some key differences. The largest PANC1 3D homoculture nodules were smaller on average than the largest nodules grown above embedded fibroblasts. (Figure 17 [D]) This could be a sign of increased proliferation, or some sort of non-contact mediated aggregation in EOCs. The largest homoculture nodules were also more circular on average than the largest nodules grown above embedded stroma. (Figure 17 [E]) Drug resistant PDAC cells acquire a more invasive phenotype, and the reduced circularity of large co-culture nodules relative to stable 3D
homoculture nodules could be a sign of cells breaking away from EOCs and invading into or onto the Matrigel bed. Difficulties distinguishing cell lines in the current model hindered our ability to quantitively differentiate certain results of nodules grown in the EOC model, but this platform might be greatly improved through small adjustments to the experimental design such as fluorescent labeling of cell lines or the application of time-lapse microscopy to capture dynamic responses to paracrine signals.

**Figure 17:** EOC data. 5x darkfield image data of PANC1 3D homoculture nodules [A], PANC1-MRC5 EOC nodules [B] and PANC1-PSC EOC nodules [C] after 7 days of growth. Scale bars = 400um [D]: Average nodule size for the largest 2.5%, 5%, and 10% of MRC5 EOC (purple) PSC-EOC (blue) and PANC1 homoculture (red) objects in each EOC condition. Co-culture nodules are consistently larger than homoculture nodules. [E]: Nodule circularity for each condition as measured by actual object perimeter divided by equivalent circular circumference. We find homoculture nodules are consistently more circular than either EOC condition.
5.1 Drug delivery is enhanced when paired with PDT pretreatment in 3D PANC1 spheroid homoculture

We exposed PANC1 spheroids to a combination of PDT pretreatment followed by a dose of Doxorubicin (DOX). Fluorescence image data obtained after treatment suggests enhanced penetration of DOX into PDT pre-treated spheroids. (Figure 18) These pretreated spheroids exhibit greater DOX channel intensity upon imaging when compared to spheroids that received DOX only. (Figure 18, [B]) This increased intensity is indicative of enhanced delivery of DOX to the center of PDT pretreated spheroids.
**Figure 18:** Homoculture drug penetration data. [A]: 40x fluorescence microscopy comparison of doxorubicin intensity in a nodule that was first pre-treated with BPD combined with a 10J PDT dose, followed 24 hours later by the administration of Doxorubicin (Left) vs. a nodule that received only doxorubicin (Right). Scale bars = 100um [B]: Comparison of mean doxorubicin intensity in PDT pretreated nodules vs. nodules receiving only doxorubicin. Nodules which have received PDT pretreatment have nearly 30% greater doxorubicin channel intensity suggesting increased drug delivery following PDT.

Plotting mean intensity as a function of nodule size further highlights the differential response. (Figure 19) Larger spheroids with no pretreatment appear to have decreasing DOX penetration with size. On the other hand, all pre-treated spheroids indicate strong DOX penetration. Further supporting enhanced drug delivery in PDT pretreated homoculture spheroids.
**Figure 19:** Plot of average DOX intensity versus nodule diameter. Here PDT pretreatment consistently enhances DOX intensity.

5.2 PDT pretreatment of AOC aggregates proves inconclusive

We repeated the drug delivery experiment on AOC platform aggregates. While image data suggests qualitative evidence that doxorubicin delivery might be enhanced by the stromal depletion, quantifying these results proved elusive. (Figure 20) There are a number of problems encountered while utilizing AOC aggregates in this manner. The actual final composition of aggregate nodules is difficult to discern in part because not all of the cells in any given experimental well are aggregated within the primary nodule. Further, while aggregate nodules are comprised of relatively three-dimensional components, the final aggregate has a mostly planar geometry. These aggregates seem somewhat more resistant to in vitro killing and qualitatively resemble human tumors, but it remains difficult to determine their usefulness and validity as an in vitro experimental platform.
Figure 20: Confocal image data of AOC aggregate nodules. [A]: Nodule treated only with doxorubicin. Live cell staining with Calcein (green) highlights the intermingling of fibroblasts and PANC1 nodules. Doxorubicin (red) still accumulates in the cancer nodules. [B]: Nodule pretreated with BPD combined with a 10J dose of PDT followed a day later with a dose of doxorubicin. Qualitative evidence of stromal depletion and enhanced drug delivery seems promising, but quantification proves elusive. Further, the 2D aggregation structure of AOC nodules limits the viability of AOC aggregates for drug penetration studies.

Scale bars = 400μm
CHAPTER 6

CONCLUSIONS AND FUTURE WORK

Juxtacrine signaling mediates in vitro PANC1 nodule aggregation. When nodules are physically separated from embedded fibroblasts limiting interactions only to paracrine signaling, as in the EOC model, they largely remain distributed across the Matrigel bed. However, when fibroblasts are free to physically attach to PANC1 nodules, as in the AOC model, the nodules are physically pulled into macroscopic aggregates.

These AOC aggregates effectively recapitulate core aspects of desmoplasia found in human PDAC tumors in a versatile three-dimensional in vitro experimental platform. Specifically, post-contraction we see tumor nodules enmeshed in dense fibrotic tissues on ECM. From the contractile event we can glean insight into the mechanics of these tumor fibroblast interactions. Here we apply time-lapse image analysis and PIVlab, an open-source particle image velocimetry tool, to analyze differential fibroblast contractility between various cell lines.
We compared MyCAF-like fibroblasts (MRC5s) with iCAF-like fibroblasts (PSCs) and found that MRC5 aggregates are larger and demonstrate a much greater shift in object size and clustering than PSC aggregates, with primary nodules encompassing the majority of cells in the well. Further MRC5 AOCs result in a nearly twice as strong contractile response as PSC AOCs. MyCAF interactions are known to be mediated by juxtacrine signaling, and the differential response between MRC5 and PSC AOCs suggests fibroblast contractility, and the intercellular forces induced by stromal crosstalk, are mediated by juxtacrine interactions within the PDAC tumor microenvironment.

We then applied the same experimental system to show that PDAC cells which have acquired drug-resistance exhibit a muted contractile interaction with fibroblasts when compared with drug naïve PDAC cells. This coincides with mRNA analysis findings that a consequence of acquired drug resistance in PDAC is reduced expression of genes which code for cell-cell adhesion. These findings are consistent with juxtacrine mediated fibroblast contractility. Drug resistant PDAC cells are unable to form the physical connections necessary to initiate the contractile aggregation observed when drug naïve PDAC cells are cultured with MyCAFs. Interestingly, we find post-aggregation activity in drug resistant PDAC AOCs to be much greater. Drug resistance is linked to acquisition of a more invasive phenotype, and stromal crosstalk is also implicated in acquired EMT and metastasis. Further quantification of this increased activity could help to understand any synergistic relationship that may exist between stroma and drug resistant PDAC but doing so with the AOC platform would likely
require labeling of at least one of the cell lines in order to differentiate motile single cells under such dynamic conditions.

Collectively these velocimetric AOC experiments demonstrate the usefulness of the platform for differentiating juxtacrine mediated contractile response in vitro. Here we’ve applied this technology to differentiating iCAF-like vs MyCAF-like as well as drug resistant vs drug naïve juxtacrine interactions, however, we could further apply a similar experimental design from an alternative perspective. For instance, we might measure the effects of parameters such as gene suppression on fibroblast contractility.

After nodules have aggregated, we are left with a complex intermingling of 3D cancer cell nodules and stroma. We utilize the aggregate nodules in a less than successful effort to quantify Stromal depletion by PDT and resulting differential drug penetration. Quantitative analysis of both stromal depletion and drug penetration was inconclusive, and we have to ask if the qualitative recapitulation of tumor-stroma comingling in human cancer doesn’t belie a fundamentally flawed two-dimensional geometry where cancer cells remain overly exposed to in vitro killing methods. Despite inconclusive stromal depletion and drug penetration data in AOC experiments, we stumbled on an interesting finding in the corresponding 3D homoculture experiments. Here, PDT pretreatment enhances drug delivery into PDAC spheroids grown in homoculture. This is promising evidence of one potential role PDT might play in a multipronged approach to PDAC treatment.
EOC experiments were intended to explore paracrine signaling between tumor cells and fibroblasts. However, the primary result from these experiments supports the finding that physical desmoplastic interactions, such as fibroblast contractility and in vitro nodule aggregation, are primarily mediated by juxtacrine signaling in the tumor microenvironment. Unfortunately, evidence of paracrine signaling in EOCs remains somewhat elusive. Still, there were a handful of promising findings in EOC experiments that may inform future implementations of similar experimental design. Images of co-cultures after seven days of spheroid growth suggest very different cell culture environments in EOCs when compared with PANC1 homocultures, and quantitatively, EOC nodules are both larger and less circular than homoculture spheroids.

There are several ways we might address the shortcomings of EOC experiments to better explore paracrine tumor-fibroblast interactions. Time-lapse imaging of EOCs could highlight dynamic changes, and potentially inform our understanding of in vitro spheroid self-assembly under differing cell culture conditions. Fluorescent labeling of cells in EOCs would have also made it possible to differentiate cells lines. This for instance, could inform whether or not PDAC cells are undergoing EMT and invading into or across the top of the Matrigel, which and could be evidence of paracrine mediated PDAC invasion.

In all, the AOC experiments demonstrate a robust platform for evaluating juxtacrine mediated fibroblast contractility, as well as compelling dynamic regimes that may prove useful for further exploring complexities of the tumor microenvironment. EOC experiments were promising but could benefit from refinement. AOC and EOC platforms could both be
enhanced by fluorescent cell labeling. While drug delivery experiments with AOCs were less successful, they did provide evidence of enhanced drug delivery with PDT pretreatment in homoculture spheroids while also highlighting a space for further development of novel complex co-culturing methods for human tissue research. Time lapse microscopy and complex three-dimensional cell culture techniques, along with developments in low cost and accurate computational image analysis platforms all proved to be compelling technologies for studying the complexities of the tumor microenvironment in vitro while offering significant opportunity for further development and implementation.
REFERENCES


