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Project Summary

Breast cancer is the second cause of mortalities among women in the United States. Although there has been a major thrust in cancer therapeutic research, the majority of clinical studies fail due to not taking into account the complex interactions in the tumor-stroma microenvironment, such as cell-cell and cell-ECM, that generally up-regulate drug resistance. Preclinical studies are often being conducted within 2D mono-layer assays of mono-culture of cells, which do not represent the tumor microenvironment inside the body. There is ample evidence suggesting that stromal cells impart drug resistance to cancer cells but there are few models that can recapitulate the tumor-stroma microenvironment with multi-parametric control to study this phenomenon. Although *in vivo* models enable organism-level studies, key parameters, such as cell- and ECM-types, cell-cell signaling, and drug transport, prove challenging to control. This has created a need to develop a model that can precisely control these parameters to study such behavior *in vitro*. Current models do recapitulate certain aspects of the tumor-stroma, such as the primary tumor region, but without a stromal region, vasculature, and 3D matrices. This has created a disruption in cell-cell and cell-ECM interactions that may affect drug responsiveness. To that end, this proposal describes the application of a developed tumor-stroma microfluidic platform investigating pharmacological effects within a co-culture system of breast cancer and stromal cells. The developed model is a microengineered device featuring well-defined spatial organization of cells and precise control of biochemical cues containing localized tumor constructs, 3D hydrogel matrices, and media perfusion channels. The goal of this project is to validate and apply a three-dimensional tumor-stroma platform for fundamental tumor-stroma interactions and pharmacological studies. Aim 1 focuses on the investigation of how fibroblasts within the tumor-stroma system affect the growth and invasiveness of the cancer. Aim 2 focuses on the communication between fibroblasts and cancer cells. We will investigate the CXCL12/CXCR4 pathway and its role in doxorubicin resistance. AMD3100, a CXCR4 inhibitor, will be introduced to the tumor-stroma microenvironment to enhance the effectiveness of doxorubicin. Finally, Aim 3 will utilize the model to study drug transport. Fibroblasts deposit ECM into the stroma limiting the effectiveness of drug delivery. We intend to create a cell-free remodeled stroma to isolate the physical alterations from cell-cell signaling for the study of drug penetration. The results from this project will validate our platform and provide expansive use of it for fundamental cancer biology and pharmacological studies.

Project Narrative

Breast cancer is the second leading cause of death among American women highlighting a need to fabricate targeted platforms recapitulating the tumor microenvironment for aggressive development of cancer therapeutics. The goal of this proposal is to develop and validate our complex tumor-stroma model describing the role of fibroblasts in cancer invasion and pharmacological studies. This should translate into a novel drug-screening tool utilizing a physiologically relevant 3D tumor microenvironment. The results from this project will likely affect the drug development pipeline by providing an alternative and complementary testing environment for current used models.

1. Thoma, C.R., Zimmermann, M., Agarkova, I., Kelm, J.M. & Krek, W. 3D cell culture systems modeling tumor growth determinants in cancer target discovery. *Adv Drug Deliv Rev* **69-70**, 29-41 (2014).
2. Lee, J.M.*, et al.* A three-dimensional microenvironment alters protein expression and chemosensitivity of epithelial ovarian cancer cells in vitro. *Laboratory Investigation; a Journal of Technical Methods and Pathology* **93**, 528-542 (2013).
3. McMillin, D.W., Negri, J.M. & Mitsiades, C.S. The role of tumour-stromal interactions in modifying drug response: challenges and opportunities. *Nat Rev Drug Discov* **12**, 217-228 (2013).
4. Paraiso, K.H.T. & Smalley, K.S.M. Fibroblast-mediated drug resistance in cancer. *Biochemical Pharmacology* **85**, 1033-1041 (2013).
5. dit Faute, M.A.*, et al.* Distinctive alterations of invasiveness, drug resistance and cell-cell organization in 3D-cultures of MCF-7, a human breast cancer cell line, and its multidrug resistant variant. *Clin Exp Metastasis* **19**, 161-168 (2002).
6. Kola, I. The state of innovation in drug development. *Clin Pharmacol Ther* **83**, 227-230 (2008).
7. Weaver, V.M.*, et al.* Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol* **137**, 231-245 (1997).
8. Yip, D. & Cho, C.H. A multicellular 3D heterospheroid model of liver tumor and stromal cells in collagen gel for anti-cancer drug testing. *Biochem Biophys Res Commun* **433**, 327-332 (2013).
9. Loessner, D.*, et al.* Bioengineered 3D platform to explore cell-ECM interactions and drug resistance of epithelial ovarian cancer cells. *Biomaterials* **31**, 8494-8506 (2010).
10. Chen, L.*, et al.* The enhancement of cancer stem cell properties of MCF-7 cells in 3D collagen scaffolds for modeling of cancer and anti-cancer drugs. *Biomaterials* **33**, 1437-1444 (2012).
11. Bray, L.J.*, et al.* Multi-parametric hydrogels support 3D in vitro bioengineered microenvironment models of tumour angiogenesis. *Biomaterials* **53**, 609-620 (2015).
12. Patel, D.*, et al.* Microfluidic co-cultures with hydrogel-based ligand trap to study paracrine signals giving rise to cancer drug resistance. *Lab Chip* **15**, 4614-4624 (2015).
13. Engel, B.J.*, et al.* Multilayered, Hyaluronic Acid-Based Hydrogel Formulations Suitable for Automated 3D High Throughput Drug Screening of Cancer-Stromal Cell Cocultures. *Adv Healthc Mater* **4**, 1664-1674 (2015).
14. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. *Nat Rev Cancer* **6**, 392-401 (2006).
15. Ivey, J.W., Bonakdar, M., Kanitkar, A., Davalos, R.V. & Verbridge, S.S. Improving cancer therapies by targeting the physical and chemical hallmarks of the tumor microenvironment. *Cancer Lett* (2015).
16. Luo, H., Tu, G., Liu, Z. & Liu, M. Cancer-associated fibroblasts: a multifaceted driver of breast cancer progression. *Cancer Lett* **361**, 155-163 (2015).
17. Loeffler, M., Kruger, J.A., Niethammer, A.G. & Reisfeld, R.A. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *Journal of Clinical Investigation* **116**, 1955-1962 (2006).
18. Santos, A.M., Jung, J., Aziz, N., Kissil, J.L. & Pure, E. Targeting fibroblast activation protein inhibits tumor stromagenesis and growth in mice. *The Journal of clinical investigation* **119**, 3613-3625 (2009).
19. Li, L. & Lu, Y. Optimizing a 3D Culture System to Study the Interaction between Epithelial Breast Cancer and Its Surrounding Fibroblasts. *Journal of Cancer* **2**, 458-466 (2011).
20. Orimo, A.*, et al.* Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* **121**, 335-348 (2005).
21. Soon, P.S.*, et al.* Breast cancer-associated fibroblasts induce epithelial-to-mesenchymal transition in breast cancer cells. *Endocr Relat Cancer* **20**, 1-12 (2013).
22. Teicher, B.A. & Fricker, S.P. CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clinical cancer research : an official journal of the American Association for Cancer Research* **16**, 2927-2931 (2010).
23. Domanska, U.M.*, et al.* A review on CXCR4/CXCL12 axis in oncology: no place to hide. *Eur J Cancer* **49**, 219-230 (2013).
24. Domanska, U.M.*, et al.* CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. *Neoplasia* **14**, 709-718 (2012).

25. Azab, A.K., *et al.* CXCR4 inhibitor AMD3100 disrupts the interaction of multiple myeloma cells with the bone marrow microenvironment and enhances their sensitivity to therapy. *Blood* **113**, 4341-4351 (2009).
26. Marchesi, F., *et al.* Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer Res* **64**, 8420-8427 (2004).
27. Tiago, M., *et al.* Fibroblasts Protect Melanoma Cells from the Cytotoxic Effects of Doxorubicin. *Tissue Engineering Part A* **20**, 2412-2421 (2014).
28. Weigelt, B., Ghajar, C.M. & Bissell, M.J. The need for complex 3D culture models to unravel novel pathways and identify accurate biomarkers in breast cancer. *Adv Drug Deliv Rev* **69-70**, 42-51 (2014).
29. Smalley, K.S., Lioni, M. & Herlyn, M. Life isn't flat: taking cancer biology to the next dimension. *In Vitro Cell Dev Biol Anim* **42**, 242-247 (2006).
30. Wang, S.J., Saadi, W., Lin, F., Minh-Canh Nguyen, C. & Li Jeon, N. Differential effects of EGF gradient profiles on MDA-MB-231 breast cancer cell chemotaxis. *Exp Cell Res* **300**, 180-189 (2004).
31. Saadi, W., Wang, S.J., Lin, F. & Jeon, N.L. A parallel-gradient microfluidic chamber for quantitative analysis of breast cancer cell chemotaxis. *Biomed Microdevices* **8**, 109-118 (2006).
32. Buchanan, C.F., *et al.* Three-dimensional microfluidic collagen hydrogels for investigating flow-mediated tumor-endothelial signaling and vascular organization. *Tissue Eng Part C Methods* **20**, 64-75 (2014).
33. Petersen, O.W., Rønnov-Jessen, L., Howlett, A.R. & Bissell, M.J. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proceedings of the National Academy of Sciences of the United States of America* **89**, 9064-9068 (1992).
34. Zaman, M.H., *et al.* Migration of tumor cells in 3D matrices is governed by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc Natl Acad Sci U S A* **103**, 10889-10894 (2006).
35. Zervantonakis, I.K., *et al.* Three-dimensional microfluidic model for tumor cell intravasation and endothelial barrier function. *Proc Natl Acad Sci U S A* **109**, 13515-13520 (2012).
36. Farahat, W.A., *et al.* Ensemble analysis of angiogenic growth in three-dimensional microfluidic cell cultures. *PLoS One* **7**, e37333 (2012).
37. Mosadegh, B., Saadi, W., Wang, S.J. & Jeon, N.L. Epidermal growth factor promotes breast cancer cell chemotaxis in CXCL12 gradients. *Biotechnol Bioeng* **100**, 1205-1213 (2008).
38. Zervantonakis, I., *et al.* Concentration gradients in microfluidic 3D matrix cell culture systems. *International Journal of Micro-Nano Scale Transport* **1**, 27-36 (2010).
39. Kim, B.J., *et al.* Cooperative roles of SDF-1alpha and EGF gradients on tumor cell migration revealed by a robust 3D microfluidic model. *PLoS One* **8**, e68422 (2013).
40. Jeon, J.S., Zervantonakis, I.K., Chung, S., Kamm, R.D. & Charest, J.L. In vitro model of tumor cell extravasation. *PLoS One* **8**, e56910 (2013).
41. Sung, K.E., *et al.* Transition to invasion in breast cancer: a microfluidic in vitro model enables examination of spatial and temporal effects. *Integr Biol (Camb)* **3**, 439-450 (2011).
42. Sung, K.E., *et al.* Understanding the impact of 2D and 3D fibroblast cultures on in vitro breast cancer models. *PLoS One* **8**, e76373 (2013).
43. Cox, M.C., Reese, L.M., Bickford, L.R. & Verbridge, S.S. Toward the Broad Adoption of 3D Tumor Models in the Cancer Drug Pipeline. *ACS Biomaterials Science & Engineering* **1**, 877-894 (2015).
44. Goswami, S., *et al.* Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res* **65**, 5278-5283 (2005).
45. Wyckoff, J., *et al.* A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* **64**, 7022-7029 (2004).
46. Palmieri, D., *et al.* Vorinostat Inhibits Brain Metastatic Colonization in a Model of Triple-Negative Breast Cancer and Induces DNA Double-Strand Breaks. *Clinical Cancer Research* **15**, 6148-6157 (2009).

47. Strobl, J.S., Nikkhah, M. & Agah, M. Actions of the anti-cancer drug suberoylanilide hydroxamic acid (SAHA) on human breast cancer cytoarchitecture in silicon microstructures. *Biomaterials* **31**, 7043-7050 (2010).
48. Nikkhah, M*., et al.* MCF10A and MDA-MB-231 human breast basal epithelial cell co-culture in silicon micro-arrays. *Biomaterials* **32**, 7625-7632 (2011).
49. Bhowmick, N.A., Neilson, E.G. & Moses, H.L. Stromal fibroblasts in cancer initiation and progression. *Nature* **432**, 332-337 (2004).
50. Sadlonova, A*., et al.* Breast fibroblasts modulate epithelial cell proliferation in three-dimensional in vitro co-culture. *Breast cancer research : BCR* **7**, R46-59 (2005).
51. Wang, X*., et al.* A complex 3D human tissue culture system based on mammary stromal cells and silk scaffolds for modeling breast morphogenesis and function. *Biomaterials* **31**, 3920-3929 (2010).
52. Luhr, I*., et al.* Mammary fibroblasts regulate morphogenesis of normal and tumorigenic breast epithelial cells by mechanical and paracrine signals. *Cancer Lett* **325**, 175-188 (2012).
53. Sabeh, F., Shimizu-Hirota, R. & Weiss, S.J. Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. *J Cell Biol* **185**, 11-19 (2009).
54. Kojima, Y*., et al.* Autocrine TGF-β and stromal cell-derived factor-1 (SDF-1) signaling drives the evolution of tumor-promoting mammary stromal myofibroblasts. *Proceedings of the National Academy of Sciences* **107**, 20009-20014 (2010).
55. Ito, A., Nakajima, S., Sasaguri, Y., Nagase, H. & Mori, Y. Co-culture of human breast adenocarcinoma MCF-7 cells and human dermal fibroblasts enhances the production of matrix metalloproteinases 1, 2 and 3 in fibroblasts. *Br J Cancer* **71**, 1039-1045 (1995).
56. Olsen, C.J., Moreira, J., Lukanidin, E.M. & Ambartsumian, N.S. Human mammary fibroblasts stimulate invasion of breast cancer cells in a three-dimensional culture and increase stroma development in mouse xenografts. *BMC Cancer* **10**, 444 (2010).
57. Comito, G*., et al.* Cancer-associated fibroblasts and M2-polarized macrophages synergize during prostate carcinoma progression. *Oncogene* **33**, 2423-2431 (2014).
58. Hansen, R.K. & Bissell, M.J. Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones. *Endocrine-Related Cancer* **7**, 95-113 (2000).
59. Bissell, M.J., Radisky, D.C., Rizki, A., Weaver, V.M. & Petersen, O.W. The organizing principle: microenvironmental influences in the normal and malignant breast. *Differentiation* **70**, 537-546 (2002).
60. Green, C.E*., et al.* Chemoattractant Signaling between Tumor Cells and Macrophages Regulates Cancer Cell Migration, Metastasis and Neovascularization. *PLoS ONE* **4**, e6713 (2009).
61. Chou, T.C. & Talalay, P. Analysis of Combined Drug Effects - a New Look at a Very Old Problem. *Trends Pharmacol Sci* **4**, 450-454 (1983).
62. Ashton, J.C. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method-Letter. *Cancer Research* **75**, 2400-2400 (2015).
63. Jain, R.K. Delivery of molecular and cellular medicine to solid tumors. *Adv Drug Deliv Rev* **64**, 353-365 (2012).
64. Mao, Y., Keller, E.T., Garfield, D.H., Shen, K. & Wang, J. Stromal cells in tumor microenvironment and breast cancer. *Cancer Metastasis Rev* **32**, 303-315 (2013).
65. Xing, F., Saidou, J. & Watabe, K. Cancer associated fibroblasts (CAFs) in tumor microenvironment. *Frontiers in Bioscience (Landmark Edition)* **15**, 166-179 (2010).
66. Provenzano, P.P*., et al.* Collagen density promotes mammary tumor initiation and progression. *BMC Med* **6**, 11 (2008).
67. Provenzano, P.P*., et al.* Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med* **4**, 38 (2006).
68. Swartz, M.A. & Lund, A.W. Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat Rev Cancer* **12**, 210-219 (2012).

69. Wu, M. & Swartz, M.A. Modeling Tumor Microenvironments In Vitro. *Journal of Biomechanical Engineering* **136**, 021011-021011 (2014).
70. Cross, V.L.*, et al.* Dense type I collagen matrices that support cellular remodeling and microfabrication for studies of tumor angiogenesis and vasculogenesis in vitro. *Biomaterials* **31**, 8596-8607 (2010).
71. Brightman, A.O.*, et al.* Time-lapse confocal reflection microscopy of collagen fibrillogenesis and extracellular matrix assembly in vitro. *Biopolymers* **54**, 222-234 (2000).
72. Ong, S.M.*, et al.* Engineering a scaffold-free 3D tumor model for in vitro drug penetration studies. *Biomaterials* **31**, 1180-1190 (2010).
73. Peela, N.*, et al.* A three dimensional micropatterned tumor model for breast cancer cell migration studies. *Biomaterials* **81**, 72-83 (2015).

Laboratory: Dr. Mehdi Nikkhah (sponsor) has a well-equipped 1000 square feet laboratory of four working benches and three dedicated rooms to microscopy, chemistry, and cell culture in addition to 1700 square feet of shared lab space on the first floor of the Intergrated Technology and Science Building 1 at the Arizona State University Tempe Campus. The microscopy room contains a fluorescent microscope equipped with an apparatus for optical sectioning to image in three-dimensions. Additionally, there is a custom-built microscope incubator to be used for the live-imaging of cells. The lab has access to core facilities that provides autoclaves, dishwashers, PCR, western blots, confocal microscopy, SEM, and histology. I myself have my own desk and workbench that has access to statistical, image analysis, manuscript preparation, and graphical editing software. Additionally, there are networked scanners, printers, and back-up storage.

Resources:

Mouneimne Lab: Dr. Mouneimne is a collaborator and on my committee. His laboratory is located in Tucson, which is an hour and a half drive. We have been collaborating for the past year and frequently send data and analysis back and forth by email. Additionally, we use overnight shipping to send samples to each other for further analysis. His lab has a Nikon Eclipse Ti-E Inverted Microscope equipped with widefield fluorescence, Phase Contrast, and Differential Interference Contrast (DIC) imaging capabilities which are powered by a SOLA LED light engine which replaces traditional Arc and Metal Halide lamps for cool, stable, and UV and IR free light. This imaging system also houses a motorized transmitted light shutter for virtually delay-free switching between transmitted light and epi-fluorescence, a high quality 10X, and apochromat 20X, 60X and 100X objective lenses which include an anti-reflective nano-crystal coat to correct for chromatic aberrations and minimize excitation light which reduces photo-bleaching and cell toxicity. Also included on their Nikon Ti-E microscope is a motorized optical path changeover with two available photo ports to which we have installed a Hamamatsu Orca Flash 4.0 V2 Digital CMOS camera and a Photometrics Evolve 512 EMCCD camera. The Orca features on-chip lens technology for high Quantum Efficiency, and the lowest read noise of any other CCD or sCMOS camera for top performing signal to noise ratio. This allows for the detection of signal at low light levels to compare small changes in intensity and discriminate small signals amid large backgrounds. The Orca is also designed with 4.0 megapixels at 6.5 μm × 6.5 μm each which allows for highly detailed and resolved image acquisition.

Additionally their lab maintains stocks of a variety of cancer cell lines including: MCF7, SUM159, MDA-MB 231, BT-474, T-47D, 293T and CACO-2 cells. All of which, I have access to if I want to expand my platform to other cell lines.

Core Bioengineering Cell Culture, Microscopy and Imaging: The core facilities for bioengineering contain two laminar flow hoods, eight cell culture incubators, centrifuges, freezers, refrigerators, a fluorescence assisted cell sorter (FACS), Nikon video microscopes, and two Nikon optical microscopes, Olympus research-grade light and fluorescence microscopes. In addition, FPLC, HPLC units, dual beam UV/Vis Spectrophotometers, Raman Spectroscopy, capillary electrophoresis systems, PCR thermal cycler, and microplate spectrophotometers are available as well.

W. M. Keck Bioimaging Resource Facility: The W. M. Keck Bioimaging facility is a multi-user facility dedicated to imaging from the macroscopic to the microscopic level. Multiple instruments are housed in the laboratory. A wide a range of microscopes are available, including scanning multi-photon microscopes, laser confocal microscopes, and atomic force microscopes and any needed technical support are available at the Keck Bioimaging Center.

John M. Cowley Center for High Resolution Microscopy:

JEOL 2010F Analytical Electron Microscope fitted with a Gatan Enfian spectrometer for
EELS
FEI TECNAI f20 Environmental Electron Microscope
Philips CM200-FEG
JEOL JEM 4000EX
Applications:
High resolution imaging and spectroscopy: HREM, XEDS, EELS, STEM, HAADF
Nanodiffraction
Leroy Eyring Center for Solid State Sciences (LECSSS)

Goldwater Materials Science Laboratory (for materials characterization)

X-ray Photoelectron Spectroscopy (XPS): Kratos XSAM800; normal and angle resolved mode
Auger Electron Spectroscopy (AES): Physical Electronics SAM 590; with depth profiling facility
Secondary Ion Mass Spectrometry (SIMS): Cameca IMS 3f ion microscope Ion Beam Analysis/Rutherford Backscattering of Materials (IBeAM) using a Tandetron accelerator
X-Ray diffraction (XRD): Rigaku D/Max-IIB; glancing angle, polefigure, rocking curve
Fourier Transform Infrared Spectroscopy (FTIR): Bruker IFS 66 V/S
Atomic Force Microscopy (AFM): Digital Nanoscope III Multimode

ASU NanoFab facilities:

The NanoFab facilities contain a variety of shared laboratories including a 4,000 square foot Class 100 cleanroom that supports semiconductor wafer (up to 4 inch) and device processing. Accessories include furnaces for diffusion, annealing, oxidation, a rapid thermal processing system, evaporators, and sputtering stations for the deposition of contacts and dielectrics, and several reactive ion etchers, including a deep silicon etch tool. Facilities for computer aided design, mask making, and optical lithography are available. If necessary, very small feature sizes down to 25nm we can be patterned using a JEOL 6000SF electron beam lithography facility. A Hitachi 5700 Field Emission SEM with a resolution of ~2nm can be used to image the devices. A direct-write optical laser facility is also available and can be used for rapid turn-around prototyping of the electrode arrays. An EVG 520HE wafer bonding system will be used to enclose the implantable platform at the wafer-level with an alignment accuracy of better than a few microns. Areas of supported research include: Microelectronics, MEMS and Nano-fluidics; Wide Band Gap Semiconductors; High-K Dielectrics and Nano-magnetics.

Core Biotechniques Instructional Lab:

This Laboratory supports a course jointly offered by the Harrington Department of Bioengineering and the Department of Biology. The course provides a laboratory experience that allows Bioengineering and Biology students to engage in all levels of experimental design and analysis at the cellular level. The course introduces students to mammalian cell culture techniques, cell behavior, cell fractionation, protein analysis, video microscopy and image analysis using NIH ImageJ, biochemical assays, immunocytochemistry, and microgravity bioreactors.

Proteomics and Protein Chemistry Resource Facility:

The Proteomics and Protein Chemistry Facility (College of Liberal Arts and Sciences) is located in the Goldwater Center and staffed with two full-time personnel. Services provided by the facility include molecular mass determinations by MALDI-TOF mass spectrometry, protein sequencing by automated Edman degradation, solid phase peptide synthesis using Fmoc chemistry, and amino acid analysis. In addition, the facility maintains equipment for sample purification and analysis via HPLC and perfusion chromatography, a triple-quadrupole electrospray mass spectrometry system that can be interfaced with the HPLC system for LC-MS experiments, a capillary electrophoresis system, and a spectropolarimeter for circular dichroism (CD) measurements. Use of facility equipment by graduate students and postdoctoral researchers is encouraged, and training students how to correctly use equipment in the facility is provided free of charge. The ***core analytical facility*** is located within the protein chemistry laboratory run by the College of Liberal Arts and Sciences at ASU. The facility includes HPLC and FPLC capabilities, a dual beam UV/Vis spectophotometer, and Raman spectroscopy. It also includes 2 capillary electrophoresis systems, a microplate reader and washer with both UV and fluorescence capabilities, and a thermocycler, a goniometer with video imaging capabilities.

Equipment

The Nikkhah Laboratory is a well-equipped facility that has the capabilities to carry out the research in this training plan. This lab maintains equipment such as two biological safety cabinets, two cell culture incubators (Thermo Fisher), refrigerators, precision mass balance (Mettler Toledo), centrifuges (Thermo Fisher), oxygen plasma cleaner (Harvard Apparatus), microfluidic pump (Harvard Apparatus), ZEISS Axio Observer Z1 Fully Automated Fluorescence Microscope with Apotome2 for 3D imaging, two chemical fume hoods, computer workstations, COMSOL software for computational analysis, printers, and a -20ºC freezer.

Major Equipment in Nikkhah Lab:

- UV based photolithography system
- High precision balance
- Oxygen plasma cleaner
- Chemical fume hoods
- Cell culture biohazard safety cabinets
- Cell culture incubators
- Refrigerator/-80 °C, -20 °C freezers
- Centrifuge and Microfuges
- Multiple hotplates and magnetic stirrers
- 80 °C oven
- Fully automated ZEISS fluorescence microscope with ApoTome for 3D Z-stack imaging
- Phase contrast and bright field microscopes
- Sonicator, Sonicator bath
- Desiccator
- PH meter
- High speed computing desktops

Letters of Reference

1) **Dr. Barbara Smith**
   Assistant Professor of Bioengineering, School of Biological and Health Systems Engineering
   Arizona State University

   Relationship: PhD Dissertation Committee member

2) **Dr. Roger Kamm**
   Professor of Biological and Mechanical Engineering
   Cecil and Ida Green Distinguished Professor
   Massachusetts Institute of Technology

   Relationship: Project collaborator

3) **Dr. Ghassan Mouneimne**
   Assistant Professor, Cellular and Molecular Medicine
   University of Arizona Cancer Center

   Relationship: Project collaborator and PhD Dissertation Committee member

Additional Education Information

The Ph.D. program at the School of Biological and Health Systems Engineering of Arizona State University (ASU) is a rigorous program that gives students the opportunities to build and strengthen their future career in Biomedical Engineering. ASU offers connections to industry, hospitals, and research institutions while providing their own exciting laboratories with research in brain machine interfaces, rehabilitation, and tissue engineering. Their mission is to develop students through intense research that will yield in-depth knowledge with opportunities to teach and mentor. The school is a knowledge enterprise catering to motivated students willing to expand their capabilities. The program is divided into three major phases.

The first phase is set up to help the student choose their research field and finish up any deficiencies. The school requires 6 credit hours of core biomedical engineering courses, 6 credit hours of life sciences, 6 credit hours of quantitative courses, and 9 credit hours of technical electives. In addition, students are required to be a teaching assistant for one semester to help improve their interpersonal, mentoring, and teaching skills. By the end of the first year, the student is expected to have developed a plan of study with their chosen faculty advisor. Afterward, a committee is to be formed around the student based on the student's research and how the committee member will help the student improve. Following this, the student will take and pass a comprehensive exam based on fundamental questions from their research and courses provided by their committee members. The exam consists of a written portion and an oral portion. The written portion features 5 – 8 questions selected from the committee chair. The oral exam is a 2 hour defense based on the candidates responses to the written exam.

The next phase allows the student to finish up any remaining coursework before defending their dissertation prospectus. The prospectus should be a proposal of a well-developed idea that the candidate decided to pursue. After successfully defending their prospectus, the student will be able to file a Master in Passing (MIP) degree.

The final phase is dedicated to research and dissertation work. Once the student is near completion of the dissertation, they will schedule the defense. Students are expected to graduate within 5 years of passing the comprehensive exam.

Ira A. Fulton Schools of Engineering
ARIZONA STATE UNIVERSITY

April 12, 2016

To whom it may concern,

Please accept this letter certifying that Arizona State University considers Mr. Danh Truong to be a part of an underrepresented ethnic group that is economically and educationally disadvantaged and is qualified in the diversity student category to apply for a predoctoral fellowship in response to PA-14-148, titled "Ruth L. Kirschstein National Research Service Awards for Individual Predoctoral Fellowships to Promote Diversity in Health-Related Research (Parent F31 - Diversity)."

Mr. Truong is a Vietnamese-American whose parents emigrated from Vietnam to the United States after the Vietnam War. They did not have any belongings or money when they arrived making him and his family economically disadvantaged. In addition, Mr. Truong is the first in his family to pursue an education beyond middle school as neither of his parents made it to high school. This places him in an economically and educationally disadvantaged environment making him eligible for support under this program.

Thank you for your attention to this matter. Please do not hesitate to contact me directly at 480-727-6212 if you require additional information.

Thank you,

Keli Palmer Greenhagen
Manager, Academic Advising
Keli.Palmer@asu.edu
480-727-6212

OMB No. 0925-0001 (Rev. 08/12 Approved Through 8/31/2015)

APPLICANT BIOGRAPHICAL SKETCH

Use only for individual predoctoral and postdoctoral fellowships, dissertation research grants (R36),and Research Supplements to Promote Diversity in Health-Related Research (Admin Suppl). DO NOT EXCEED FIVE PAGES.

NAME OF APPLICANT: Danh Truong

eRA COMMONS USER NAME (credential, e.g., agency login): danh.truong

POSITION TITLE: Graduate Research Associate

EDUCATION/TRAINING *(Most applicants will begin with baccalaureate or other initial professional education, such as nursing. Include postdoctoral training and residency training if applicable. High school students should list their current institution and associated information. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	START DATE MM/YYYY	END DATE *(or expected end date)* MM/YYYY	FIELD OF STUDY
University of Texas at Arlington	B.S.	08/2009	05/2014	Biology
University of Texas at Arlington	M.S	12/2013	05/2014	Biomedical Engineer
Arizona State University	Ph.D.	08/2014	05/2018	Biomedical Engineer

NOTE: The Biographical Sketch may not exceed five pages. Follow the formats and instructions below.

A. Personal Statement

My research experience has only started four years ago but my passion for science and engineering began when I was young. I spent a majority of my childhood indulging in science education television shows and cartoons that showed me that there are endless possibilities that can be created with the human mind. I was heavily influenced by this notion and spent my younger years engaging in science activities. I participated in science fairs and contests even being awarded first place in a few of them. In college, I chose a dual-degree in Biology and Biomedical Engineering because I wanted to use my passion to develop medical technologies. When I had a chance to join someone's laboratory during my undergraduate career, I immediately met up with the professor after class and discussed my interest. My research then was focused developing biomaterials for cardiac tissue engineering. In that lab, I developed a stronger interest in translational research as well as core skills like critical thinking and data analysis. I graduated from the Hong lab with more questions than I had answers, which fueled my desire to earn a Ph.D. During my interviews for a doctoral program, I met Prof. Mehdi Nikkhah, who shared my interest in developing novel bioengineered platforms for studying fundamental biological issues (e.g. heart disease, cancer). Shortly afterward, I joined his lab to undergo a rigorous training program that has motivated me to pursue excellent research. After 1.5 years in his lab, I have worked on three manuscripts where two have been published and one is being reviewed. I developed two tumor models which enhanced my understanding of cancer and ability to perform independent research. This has given me the confidence to pursue the proposed studies in this training plan. Furthermore, I performed extensive preliminary studies using the previously developed models to develop a solid rationale for the proposed studies. After my training, I plan on becoming a career investigator at a research university to undergo exciting translational research and to mentor and motivate undergraduates from all cultural backgrounds toward science. For these goals, I am working one on one with underrepresented students in science, technology, engineering, and math (STEM) to help them with their research projects. Furthermore, I am engaging in education outreach to schools around the Phoenix area. Finally, I intend to establish validated *in vitro* cancer invasion platforms for the next generation of cancer research. This proposal contains the beginning of a long validation process of our developed microfluidic model for recapitulating cancer invasion.

B. Positions and Honors

ACTIVITY/ OCCUPATION	START DATE (mm/yy)	END DATE (mm/yy)	FIELD	INSTITUTION/ COMPANY	SUPERVISOR/ EMPLOYER
Research Assistant	10/12	06/14	Biomedical Engineer	UT Arlington	Dr. Yi Hong
Graduate Research Associate	08/14	Present	Biomedical Engineer	Arizona State University	Dr. Mehdi Nikkhah

Academic and Professional Honors

PTSA Student Scholarship 2009
Top 10% Award 2009-2013
Outstanding Freshman Award 2009-2013
Dean's Fellowship 2014-2018
ASU Block Funding Grant 2015
GPSA Travel Grant 2015
GPSA Jumpstart Research Grant 2016
First place Poster Presentation Award in Molecular, Cellular, and Tissue Bioengineering Symposium 2016

Memberships in Professional Societies

Biomedical Engineering Society
Tau Beta Pi
American Society of Mechanical Engineers

C. Contributions to Science

I. Undergraduate Career:

Research papers:

Punnakitikashem, P., **Truong, D.**, Menon, J. U., Nguyen, K. T., & Hong, Y. (2014). Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts. *Acta biomaterialia,* 10(11), 4618-4628.

Gao, G., Schilling, A. F., Hubbell, K., Yonezawa, T., **Truong, D.**, Hong, Y. & Cui, X. (2015). Improved properties of bone and cartilage tissue from 3D inkjet-bioprinted human mesenchymal stem cells by simultaneous deposition and photocrosslinking in PEG-GelMA. *Biotechnology letters,* 1-7.

Presentations:

Punnakitikashem, P., **Truong, D.,** Menon, J. U., Nguyen, K. T., & Hong, Y., "Drug-releasing biodegradable elastomeric fibers for vascular engineering," UT Arlington ACES Symposium 2014, Arlington, TX, March 19, 2014

Punnakitikashem, P., **Truong, D.,** Menon, J. U., Nguyen, K. T., & Hong, Y., "Drug-releasing biodegradable elastomeric fibers for vascular engineering", UT Arlington 40th Anniverssary of Bioengineering Symposium. Arlington, TX, March 26, 2014

Anand, S., Desai, V., Vasudevan, S., Nguyen, D., Tran, M., Nang, M., Alzoghoul, N., **Truong, D**., Hong, Y., Cheng, J., Keefer, E., Romero, M., "Sensory and Motor Enrichment using Molecular Guidance Cues," Neural Interface Conferences 2014, Dallas, TX, June 22-25, 2014

II. Graduate Career:

Research papers:

Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M. (2016). A three dimensional micropatterned tumor model for breast cancer cell migration studies. *Biomaterials*, 81, 72-83.

Navaie, A.*, **Truong, D**.*, Heffernan, J., Cutts., J., Overstreet, D., Sirriani, R., Brafman, D., Vernon, B., Nikkhah, M. (2015) PNIPAAm-based Biohybrid Injectable Hydrogel for Cardiac Tissue Engineering . *Acta Biomaterialia*. *, Equal contributors

Truong, D., Puleo, J., Llave, A., Mouneimne, G., Nikkhah, M. Breast Cancer Cell Invasion into a Three Dimensional Tumor-Stroma Microenvironment. Submitted and Awaiting Revisions.

Presentations:

Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M., "A Three Dimensional Micropatterned Tumor Model to Study Breast Cancer Cell Invasion," AZBIO Awards 2015, Phoenix, AZ, October 1, 2015

Truong, D., Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," AZBIO Awards 2015, Phoenix, AZ, October 1, 2015

Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M., "A Three Dimensional Micropatterned Tumor Model to Study Breast Cancer Cell Invasion," Annual Biomedical Engineering Society Meeting, Tampa, FL, October 7-10, 2015

Truong, D., Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," Annual Biomedical Engineering Society Meeting, Tampa, FL, October 7-10, 2015

Truong, D., Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M., "Three-dimensional (3D) Invasion of Breast Cancer Cells in a Well-Defined Tumor-Stroma Platform," NanoEngineering for Medicine and Biology Conference, Houston, TX, February 20-24, 2016

Truong, D., Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," Molecular, Cellular, and Tissue Bioengineering Symposium, Tempe, AZ, April 1, 2016

Navaie, A., **Truong, D**., Heffernan, J., Cutts., J., Overstreet, D., Sirriani, R., Brafman, D., Vernon, B., Nikkhah, M. PNIPAAm-based Biohybrid Injectable Hydrogel for Cardiac Tissue Engineering. World Biomaterials Congress, Montreal, Canada, May 17-22, 2016

D. Scholastic Performance

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
	University of Texas At Arlington			University of Texas At Arlington	
	Undergraduate Record			Undergraduate Record	
2009	Intro to Bioengineering	P	2009	Art Appreciation	T
2009	Cell Molecular Biology	A	2009	Expository Writing	T
2009	General Chemistry	A	2009	Critical Thinking, Read and Writing	T
2010	Structure and Function of Organisms	A	2009	World Literature	T
2010	General Chemistry	A	2009	History Transfer	T
2010	Organic Chemistry I Lab	A	2009	Precalculus II	T
2010	Organic Chemistry I	A	2009	Beginning Spanish I	T
2010	Genetics	A	2009	Beginning Spanish II	T

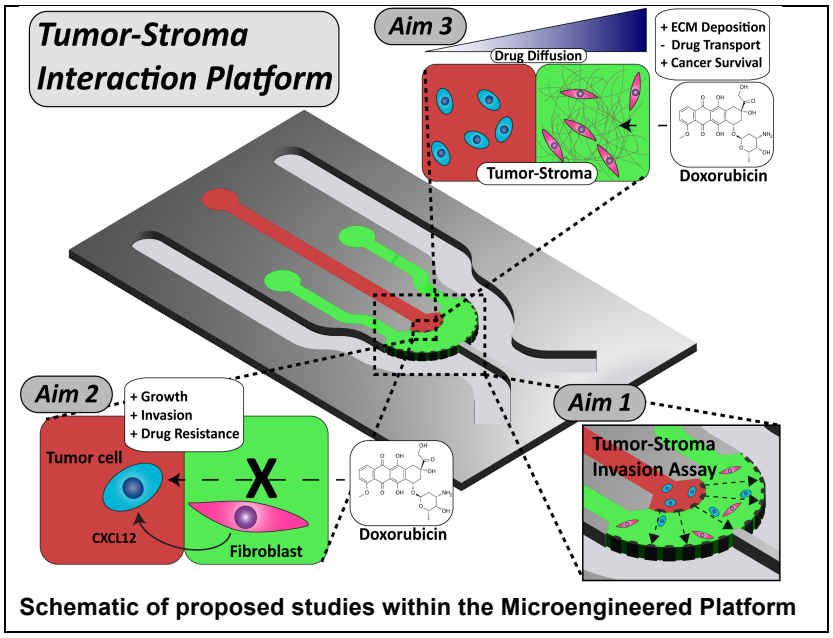
YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
2010	Organic Chemistry II	A	2010	Calculus I	T
2010	Organic Chemistry II Lab	A	2009	Martial Arts	P
2010	General Technical Physics I	A	2009	Govt of US	B
2011	General Microbiology	A	2010	State and Local Govt	A
2011	General Technical Physics II	A	2010	Honors Calculus II	A
2011	Advanced Topics in Chemistry	A	2010	History of US	A
2011	Immunobiology	A	2010	Calculus III	A
2011	Biochemistry	B	2011	Professional and Technical Comm	A
2012	Human Physiology	B	2011	Diff Equations and Linear Algebra	A
2012	Inorganic Chemistry	B	2011	Statics and Dynamics	A
2012	Introduction to Virology	A	2011	Intro to Programming	A
2013	Biometry	A	2011	Circuit Analysis	A
			2011	History of US	A
			2011	Thermodynamics	A
			2012	Fluid Mechanics	B
			2012	Experimental Methods and Measurements	A
			2012	Fundamentals of Bioengineering	A
			2012	Tissue Engineering	B
	University of Texas At Arlington		2012	Polymers in BME	A
	Graduate Record		2012	Linear Systems	B
2014	Methods in Molecular Microbiology	A	2012	Elementary Statistics	B
			2013	Directed Research in BE	P
			2013	Nanobiomaterials	A
			2013	Neural Engineering	A
			2013	Tissue Engineering Lab	B
			2013	Beginning Korean	
				University of Texas At Arlington	
				Graduate Record	
			2013	Seminar in Bioengineering	P
			2013	Directed Research in Bioengineering	P
			2013	Transport Phenomena	A
			2013	Medical Imaging	B
			2013	Biomaterials and Blood compatibility	A
			2013	Laboratory Principles	B
			2014	Directed Research in Bioengineering	P
			2014	Biological Materials, Mechanics and Processes	A
			2014	Process Control in Biotechnology	A
			2014	Biomaterials Living System Interactions	A

YEAR	SCIENCE COURSE TITLE	GRADE	YEAR	OTHER COURSE TITLE	GRADE
	Arizona State University			Arizona State University	
2015	Biochemistry of Cancer	A	2014	Seminar in Biomedical Engineering	P
			2015	Seminar in Biomedical Engineering	P
			2015	Seminar in Biomedical Engineering	P
			2016	Seminar in Biomedical Engineering	P

University of Texas at Arlington: Follows an A,B,C,D,F grading system. C and P (i.e. when there is no grade) is passing. T denotes transfer credit.

Arizona State University: Follows an A,B,C,D,F grading system. C and P (i.e. when there is no grade is passing.

SPECIFIC AIMS



Schematic of proposed studies within the Microengineered Platform

Breast cancer is the second cause of mortalities among women in the United States. Although there has been a major thrust in cancer therapeutic research, ***the majority of clinical studies fail due to not taking into account the complex interactions in the tumor-stroma microenvironment, such as cell-cell and cell-ECM, that generally up-regulate drug resistance.*** Preclinical studies are often being conducted within 2D mono-layer assays of mono-culture of cells, which do not represent the tumor microenvironment inside the body. Moreover, *in vivo* models enable organism-level studies but key parameters, such as cell- and ECM-types, cell-cell signaling, and drug transport, are difficult to control. ***Therefore, there is still an unmet crucial need to develop models incorporating relevant tumor-stroma interactions for preclinical studies to improve the clinical efficacy of anti-cancer drugs.***

Previous *in vitro* models have made great strides in recapitulating the tumor microenvironment. ***Nonetheless, there is still no model that incorporates well-controlled 3D tumor-stroma regions integrated with perfusable vasculature to enable physiologically relevant modeling of cell-cell, cell-ECM, and drug interactions.*** For instance, different platforms have compartmentalized co-culture of different cells, but there have been *absence of perfusable channels (resembling vascular networks)* to simulate drug transport within the tumor-stroma entities. Moreover, models that do enable drug-based studies, lack the ability to co-culture cells with well-defined *3D spatial compartmentalization of cells/ECM*.

Our laboratory has recently developed a microengineered platform with defined architecture enabling in-depth and extensive studies on tumor-stroma interactions. Specifically, the microfluidic platform permitted drug transport studies and created regions of defined cell- and ECM-types that mimicked the tumor-stroma organization and composition, stiffness, and heterogeneity of the tumor. Furthermore, *decoupling of the tumor and stroma regions* allowed for defined studies with various combinations of cells and ECM components.

In this proposal, ***we aim to investigate tumor-stroma interactions involving cancer-associated fibroblasts (CAFs) and highly metastatic cancer cells.*** Specifically, we will *study 3D invasion of breast cancer cells co-cultured with CAFs* or normal fibroblasts (NFs) within the proposed microengineered platform. Next, we will *recapitulate CXCL12/CXCR4 pathway* and evaluate its influence on doxorubicin resistance. Lastly, we will *study the sole role of biophysical remodeling of the stroma,* during active invasion, to assess its effect on drug penetration and function.

Specific Aim 1: To microengineer and validate a platform with 3D co-culture of cells to assess the role of cell-cell interactions, with variable cell-cell ratios, on tumor growth and invasion. We will arrange fibroblast cells around cancer cells to recapitulate the heterogenic tumor and stromal architecture and cell/ECM compositions. We will further study how fibroblasts affect tumor growth and invasion at a single cell level.

Specific Aim 2: To recapitulate the heterotypic signaling pathway (CXCL12/CXCR4) between CAFs and cancer cells and investigate its role on anti-cancer drug resistance. We will specifically study chemotherapeutic effect of doxorubicin and subsequent role of AMD3100 to block CXCL12/CXCR4 pathway.

Specific Aim 3: To assess the role of fibroblast-induced desmoplasia in doxorubicin penetration and function. We will evaluate how biophysical remodeling of stroma affects the function and transport of doxorubicin to the tumor region.

Impact: This study has broad impacts from both technological and biological perspectives. This is the first attempt to deliver a tumor-stroma platform that can be used to manipulate cell-cell, cell-ECM, and drug interactions. The unique features of this platform enable the incorporation of physical (i.e. matrix remodeling) and biochemical (i.e. cell-cell signaling) cues within a novel and well-controlled tumor microenvironment model to enhance the efficacy of drug discovery and preclinical studies.

A. BACKGROUND AND SIGNIFICANCE

Breast cancer is the second most devastating disease among women in the United States. Despite significant advances in treatment regiments, anti-cancer drugs often fail due to lack of comprehensive preclinical studies utilizing models incorporating the complexities of the native tumor microenvironment such as the surrounding stromal and the vasculature (**Fig. 1**)[1-5]. While conventional mono-culture assays has shown great promises for preliminary screening, over 90% of anti-cancer drugs fail in the more expensive Phase III trials[6]. Numerous studies have shown that cell-cell and cell-ECM interactions, are prominent factors influencing tumor growth and progression[7]. Carcinoma cells grown in 3D matrices with stromal cells (i.e. fibroblasts, immune, and endothelial) resemble the *in vivo* phenotype and organization allowing for physiologically relevant tumor-stroma interactions[8-13]. In fact, these interactions have been demonstrated to induce complex autocrine/paracrine signaling that ultimately increase drug resistance. Thus, the stromal components of the local tumor microenvironment should be considered within preclinical studies to improve the efficacy of drug screening.

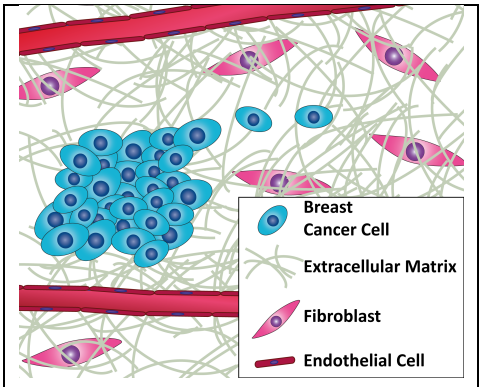


Figure 1. The several components of the breast cancer microenvironment

Stromal cells, such as fibroblasts, heavily influence cancer invasion and therapy. Fibroblast cells stand out the most, within the tumor microenvironment, and play a diverse role on cancer progression[14]. In particular, *cancer-associated fibroblasts (CAFs) contribute to drug resistance through cell-cell signaling that enhances cancer survival and invasion*[14] and *desmoplasia (i.e. deposition of ECM)*[15,16]. Several studies have shown that removal of CAFs from the *in vivo* tumor microenvironment led to enhanced efficacy of chemotherapeutics[17,18]. Furthermore, drugs that administrated on cancer cells alone had a greater reduction in tumor volume compared to co-culture with fibroblasts[19]. CAFs promote drug resistance and cancer invasion by secreting stromal cell-derived factor 1α (SDF-1α or CXCL12) to interact with the receptor, CXCR4, that up-regulate pro-survival pathways[20-23]. AMD3100 has been shown to block the receptor (CXCR4), which resulted in increased cancer responsiveness to chemotherapy[11,24-26]. *To that end, fibroblasts take a dual role in supporting cancer invasion and drug resistance through the CXCR4/CXCL12 pathway*[14,17,21,27] *as well as remodeling the microenvironment by ECM deposition to reduce drug penetration.*

There is an unmet crucial need to develop tumor microenvironment models incorporating the surrounding stroma to enable studying cell-cell and cell-matrix interactions and their subsequent roles on therapeutics efficacy of anti-cancer drugs. *In vivo* models[28,29] enable organism-level studies, but do not allow decoupled control of cell-cell and cell-ECM interactions so it is difficult to elucidate the role of each separate stromal component. On the other hand, 2D *in vitro* models[30,31] fail to recapitulate 3D heterotypic crosstalks, organization of tumor-stroma, and biomolecular transport normally found within the native tumor microenvironment. Conventional hydrogel-based matrices, such as collagen[32] and Matrigel®[33], are suitable candidates to encapsulate cancer cells within a 3D environment[34,35], enabling fundamental studies on cellular migration, angiogenesis, and proliferation[36-38]. However, macroscale conventional hydrogels often lack the precise control that is needed to create well-defined entities of cells and ECM.

Micro-scale technologies (e.g. microfluidics) integrated with hydrogel-based 3D matrices allow the study of different steps of the metastatic cascade within a well-controlled tumor microenvironment model. Recent innovations in microengineered technology have enabled studies of different aspects of metastasis such as invasion[39], intravasation[35], and extravasation[40]. Despite significant progress, mainly from Beebe[41,42] and Kamm[35,36] groups, most of the previous studies have relied on simplified tumor models. In particular, previous attempts have utilized mono-culture of cancer cells with simplistic spatial organization (i.e. separate 2D mono-layer tumor and 3D stromal region) and lack in-depth analysis and validation of cell-cell and cell-ECM interactions. For instance, Beebe group[41,42] developed a tumor model to organize fibroblasts and breast cancer cells to study the physiological crosstalks (i.e. up regulation of growth factors) but did not incorporate perfusable channels surrounding the tumor-stroma regions in lieu of a complex vasculature system to create chemotactic gradients or assess pharmacological effects of anti-cancer drugs on tumor progression. Other models enabled cancer cell migration and drug transport within a 3D matrix[39] but did not offer the ability to separately compartmentalize the tumor and stroma entities within a 3D matrix to conduct single and tissue level studies (i.e. single cell invasion, matrix remodeling). *We believe that spatial-organization of the cells for physiologically accurate cell-cell cell-ECM crosstalks are increasingly important to assess the major role of*

each component on disease progression. In this regard, there is still a lack of 3D and well defined model[43] to study the influence of cancer stromal interactions either through cell-cell signaling or biophysical alterations of stromal on efficacy (i.e. function and transport) of anti-cancer drugs.

We propose a novel microengineered tumor-stroma model that incorporates critical components of tumor-stroma interactions by (1) *co-culturing fibroblasts and breast cancer cells to investigate tumor growth and cancer invasion through modulation of cell-cell ratios,* **(2)** *recapitulating the CXCL12/CXCR4 pathway and evaluating its influence on Doxorubicin and AMD3100 drug response, and* ***(3)*** *assessing the role of matrix remodeling (i.e. biophysical cues) on drug penetration and function.*

B. INNOVATION

(1) The proposed 3D microengineered tumor-stroma model, compartmentalize cancer and stromal cells with well-defined architecture while enabling chemoattractant gradients through the peripheral 3D hydrogel-based stroma. The design of our device allows us to first localize tumor cells within a central region of the model, and then introduce the surrounding stroma, which encapsulates the tumor region and functions as a diffusion barrier for nutrients and drug transport. This enables versatile studies modulating cell-cell and cell-ECM interactions within the proposed platform.

(2) This study aims to investigate a critically relevant dialogue between cancer cells and fibroblasts, *at a single cell level*, and its subsequent role on cancer invasion and drug resistance. The proposed device enables 3D live-cell imaging to track and analyze cells within the hydrogel matrix in repose of various conditions. We can quantitatively assess cell-cell and cell-drug interactions.

(3) The diffusion studies within the proposed platform will allow us to analyze how fibroblasts change drug transport through stromal remodeling (i.e. biophysical cue) within the tumor microenvironment. Our device creates an active stromal barrier containing fibroblasts surrounding the cancer cells that can model basic physiological drug transport. Furthermore, we can introduce a high-density matrix without fibroblast to decouple the cell-cell signaling and biophysical alterations, of the stroma, for subsequent drug transport studies.

C. PRELIMINARY DATA

Fabrication of tumor-stroma platform and invasion of breast cancer cells: We have performed the necessary preliminary studies to confirm the feasibility of the proposed study. We developed a microfluidic platform that resembled the breast tumor microenvironment (**Fig. 1**) to simulate tumor-stroma interactions. The platform (**Fig. 2A**) consisted of an inner and outer semi-circle representing the tumor and stromal region respectively. There were two channels flanking the stromal region representing vascularized networks for nutrient and drug transport. COMSOL modeling was used to simulate diffusion and the results were experimentally confirmed (**Fig. 2B**). We encapsulated highly invasive SUM-159 breast cancer cells (15 million cels/mL) in a mixture of Matrigel®:Collagen-I at a ratio of 1:1 (final collagen density is 1 mg/mL) and injected the solution into the tumor region, while cell-free collagen-I (2 mg/mL) was injected into the stromal region. 50 ng/mL of EGF, denoted as (+) EGF), was utilized to stimulate cancer invasion. We observed cell migration throughout the stroma over the course of four days (**Fig. 2C**). By day 3, it was evident that (+) EGF cells invaded further (**Fig. 2D**). Furthermore, the cell morphology (**Fig. 2E**) was analyzed and correlated to EGF stimulation. We observed cytoskeletal alterations in the EGF-

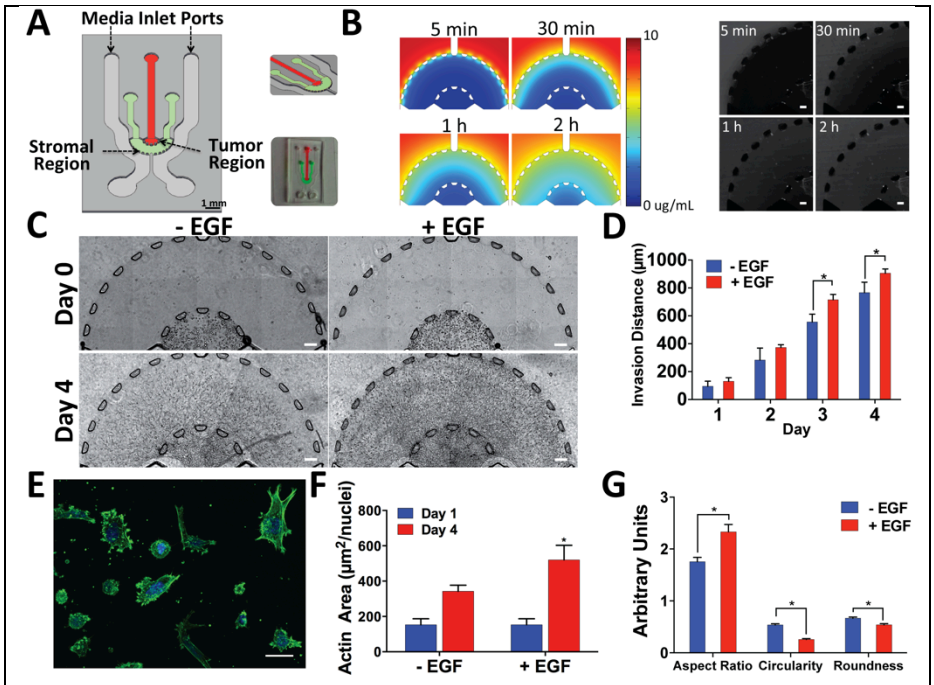


Figure 2. Design of tumor-stroma platform (A). Diffusion analysis of EGF (B) Dissemination of breast cancer cells after 4 days (C). Significant increase in invasion on days 3 and 4 due to EGF (D). F-actin image (E). Actin area and cell shape descriptors (F and G). * is significantly different.

stimulated devices, where the cells demonstrated a more invasive phenotype (**Fig. 2F** and **G**) (i.e. increased aspect ratio and reduced circularity). This preliminary study validated the platform as an invasion model by utilizing a known pathway (EGF stimulated invasion), consistent with *in vivo* studies[44,45].

Co-culture of invasive breast cancer cells with CAFs: Utilizing the developed microfluidic platform, we introduced CAFs (Low: 100,000 cells/mL or High: 1 million cells/mL) and SUM-159 (15 million cells/mL) breast cancer cell (red) within the tumor and stroma regions of the device. Over the course of three days (**Fig. 3A**), we monitored the invasive and proliferative profile of the cells in terms of migration distance and cell count. When comparing the three groups, we found that the high CAFs reduced invasion and low CAFs increased invasion (**Fig 3B** and **C**). This suggested that there are complex cell-cell interactions modulated by cell density that warrants further investigations to understand how cell-cell interactions affect tumor progression.

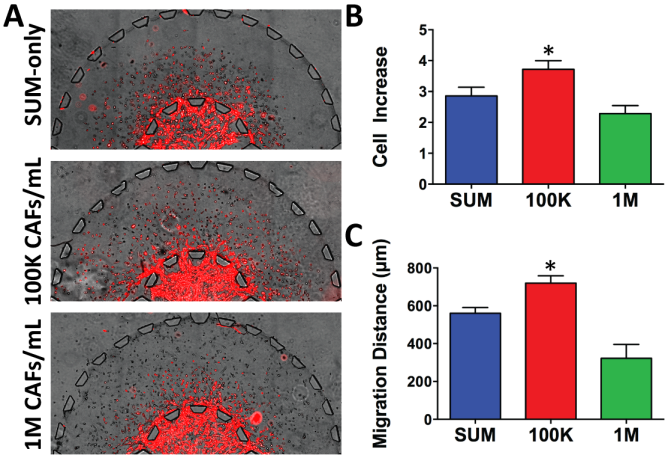


Figure 3. SUM-159 (15 million cells/mL) breast cancer cells (red) were cultured together with CAFs (100,000 cells/mL or 1 million cells/mL) on Day 3 (96 hrs) (A). CAFs ratio influenced breast cancer invasion and cell count (B and C). * is significantly different.

Effect of Suberoylanilide hydroxamic acid (SAHA) on invasive breast cancer cells: We characterized the effects of clinically approved anti-cancer drug, SAHA, on cancer cell invasion within the fabricated microfluidic device. SAHA was previously shown to inhibit the migration of cancer cells on 2D platforms and *in vivo models*[46-48]. We encapsulated 10 million cells/mL of SUM-159 within the tumor region (**Fig. 4A**). Next, we diffused different concentrations of SAHA toward the tumor region. Cell survival decreased with increasing drug concentration (**Fig. 4B**). Simultaneously, we compared cellular migration on 2D wound healing assays with the same drug concentrations (0, 2.5, 5, and 10 μM) and found no differences for 0, 2.5, and 5 μM (**Fig. 4C**). However, within our tumor-stroma platform, we were able to demonstrate significant differences between various concentrations of the drug, (**Fig. 4D**) suggesting a higher precision in testing the drug efficacy within our 3D microfluidic model compared to the traditional 2D assays. We attributed this to the nature of the 3D environment allowing for the different cell and cell-ECM interactions, as well as 3D gradients of nutrients and drug, which were not present in the 2D assay[1,3,4,27,29].

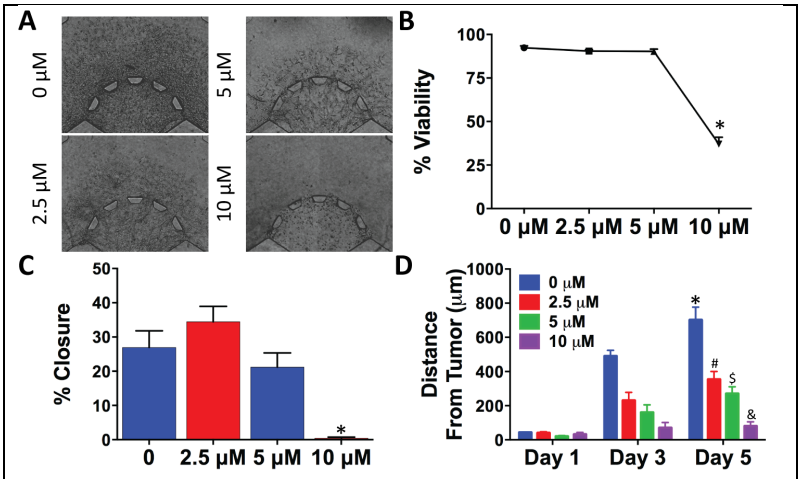


Figure 4. SUM-159 were treated with different concentrations of SAHA (A). Wound healing assay was performed with the same cells and drug concentrations. The % viability of cells within our device (B) and % closure of the wound healing assay (C) were calculated. The tumor invasion distance within the device was calculated and showed significant changes due to SAHA (D). *, #, $, and & are significantly different groups.

D. RESEARCH DESIGN AND METHODS

Aim 1. To microengineer and validate a platform with 3D co-culture of cells to assess the role of cell-cell interactions, with variable cell-cell ratios, on tumor growth and invasion.

Rationale: Breast tumor microenvironment consists of complex tissue architecture with a cascade of cell-cell and cell-matrix signaling which heavily influence disease progression. A number of studies have previously demonstrated that CAFs play a major role in cancer cell proliferation and migration[20,49-52]. CAFs have been particularly shown to increase tumor volume *in vivo* in contrast to normal fibroblasts (NFs)[20]. However, there were few studies utilizing 3D microengineered platforms, with well-defined architecture, to recapitulate the tumor-stroma microenvironment in order to precisely control cell-cell and cell-matrix interactions. Moreover, it is often overlooked how cell-cell ratios (i.e. stromal-cancer) influence cancer

invasion in a co-culture system. In this regard, elevated levels of CAFs have been utilized, as a potential prognostic marker for invasion and metastasis[14]. Therefore it is crucial to study how different ratios of fibroblasts to cancer cells influence the invasion or quiescence of cancer.

1.1 Formation of the co-culture of fibroblasts and breast cancer cells within the tumor-stroma platform with well-defined architecture: Similar to our preliminary studies, for the tumor region, we will encapsulate SUM-159 breast cancer cells within a 1:1 ratio of Matrigel:Collagen-I (**Fig. 2C**). We utilize SUM-159 as the model invasive cell line due to readiness of these cells to invade in a 3D matrix[53]. Subsequently, the devices will be placed in the incubator (37 °C, 5% CO_2, 95% relative humidity) for polymerization. Next, neutralized collagen-I (2mg/mL) with fibroblasts (**Fig. 3A**), either CAFs or NFs, will be loaded at different ratios from 1:3, 1:1, and 3:1 based (**Table 1**) on our preliminary studies and other investigations in the literature[21,54]. The cell density will be set at 3 million cells per mL. The number of fibroblast cells (CAFs or NFs) will be adjusted according to the defined ratios. Standard culture media (FBS+DMEM) will be injected into the media outlets and changed daily. The devices will be maintained within a physiological incubator during the experimentation.

Cell-Cell Ratios	SUM-159 (Tumor)	CAFs (Stroma)	NFs (Stroma)
a	3	1	×
b	1	1	×
c	1	3	×
d	3	×	1
e	1	×	1
f	1	×	3

Table 1: Experimental conditions for proposed study.

1.2 Characterization of tumor growth and cell survival: After 3 days of culture, we will stain for cytokeratin (epithelial marker, Alexa Fluor 555) and α-SMA (myofibroblast marker, Alexa Fluor 488) to differentiate the cells. We will investigate the proliferative effects of the fibroblasts on cancer cells by staining for Ki-67 (Alexa Fluor 647). In addition, the number of breast cancer cells will be counted per day to quantify the cell proliferation over time. Finally, we will assess the cell survival within the tumor-stroma platform by using an Annexin V assay on days 1 and 3.

1.3 Assessment of cancer cell invasion within the 3D matrix: We will analyze the dissemination of breast cancer cells at 48 hrs of initial culture. Briefly, m-Cherry transfected SUM-159 breast cancer cells will be prepared for this study using the same cell loading procedures and cell conditions described in 1.1. Next, the devices will be placed within a physiological microscope incubator (37 °C, 5% CO_2, 95% relative humidity). Z-stack images will be taken every 15 minutes using a 10x objective and Apotome.2 hardware (Sponsor's lab) to create high-resolution 3D movies. The software configuration will be based on: 2 x 2 binning, 3.45 um z-stack intervals, and 15 min time interval. The dissemination of cells due to cell-cell interactions will be analyzed using ImageJ and quantified for speed, distance, and persistence (i.e. how close cells move in a straight path)[30,39].

Expected Results and Contingency Plans: We have already demonstrated the successful development of defined entities for the primary tumor and the stroma confirming that these two regions are clearly separated but still connected to allow for cell migration from tumor to stroma. We expect to determine the ratios that CAFs will enhance cell proliferation and migration and that NFs will enhance apoptosis and maintain quiescence. We can modulate cell-cell ratios to develop different models of invasive or quiescent cancer. In the case that the differences in the ratio is not significant, we will modify the ratios to 1:5, 1:1, and 5:1.

Aim 2. To recapitulate the heterotypic signaling pathway (CXCL12/CXCR4) between CAFs and cancer cells and investigate its role on anti-cancer drug resistance.

Rationale: Our developed microfluidic model was used to study 3D cancer invasion in response to EGF gradient and SAHA[47,48] confirming the capabilities of the model for drug screening applications. However, it is still difficult to elucidate the effect of the cues because of the complications of isolating and modulating cell-cell interactions (e.g. fibroblasts-, macrophages-, or endothelial-cancer) in current animal models and *in vitro* platforms. We plan to expand our biological analysis to investigate heterotypic cell-cell signaling within the tumor microenvironment by looking specifically at the CXCL12/CXCR4 pathway[14]. CAFs influence the invasiveness and drug resistance of tumors and through signaling factors such as CXCL12[42,54-57]. There still does not exist an integrated tumor-stromal 3D platform to manipulate cell-cell interactions through various ratios of cancer to fibroblast cells, introducing CXCR4 drug inhibitors, and subsequent observation on CXCL12/CXCR4 pathway. Our microengineered platform allows for compartmentalization of the tumor-stroma to decouple the breast cancer cells from the fibroblasts (i.e. CAFs and NFs) with physiologically relevant spatial organization of the cells[58,59]. This arrangement allows similar drug transport from the microfluidic channels, which represents the vasculature system, through the stroma containing fibroblasts (i.e. either NFs or CAFs). We conduct this study based on different cellular ratios (**Table 1**). Next, we intend to utilize

AMD3100 to block CXCR4 to sensitize cancer cells to DOX in order to investigate how the system synergistically influence cancer drug responsiveness by assessing invasion and growth[11,24-26].

2.1 Recapitulating the CXCL12/CXCR4 pathway: In this study, we will specifically focus on CXCL12 pathway, which has been the subject of previous chemotactic studies in 3D models but not for drug resistance[39,60]. First, we will analyze production of CXCL12 (Alexa Fluor 488) through staining within the cytoplasm. Cytokeratin (Alexa Fluor 555) will be used as a contrast to differentiate between the fibroblasts and cancer epithelial cells. We will compare CXCL12 positively stained cells to negatively the fibroblasts across different conditions (**Table 1**). Next, we will quantify the amount of phosphorylated CXCR4 (Alexa Fluor 647) either by the amount of individual receptors per cell (if possible) or by positively stained breast cancer cells.

2.2 Effect of DOX on tumor growth, cell survival, and invasion: Similar to 1.2, tumor growth and survival (Annexin V assay) will be assessed under different drug conditions. We will only consider SUM-159:CAFs and SUM-159:NFs at the ratios that gave the highest and lowest tumor invasion respectively found in 1.3. We will test cell survival within our 3D platform with a broad range of DOX concentrations. Next, we will select 4 concentrations based on 0%, 25%, 50%, and 75% effectiveness (IC_0, IC_{25}, IC_{50}, IC_{75}) on reducing viability to study the tumor growth. For our invasion assays, we will utilize the wound healing assay and our microfluidic platform for the 2D (control) and 3D testing respectively. Similar to 1.3, m-Cherry transfected SUM-159 breast cancer cells will be prepared for this assay using the same cell loading procedure. At 48 hrs of culture, we will add different concentrations of DOX and assess cellular invasion. The dissemination of the cells (**Fig. 4D**) will be analyzed using ImageJ and quantified for speed, distance, and persistence.

2.3 Effect of AMD3100 on tumor growth, cell survival, and invasion: Similar to 2.2, tumor growth and survival (Annexin V assay) will be assessed under different drug conditions of AMD3100. We will use the same cell-cell ratios as used in 2.2. We will test viability of cells within our 3D platform and select 4 concentrations (IC_0, IC_{25}, IC_{50}, IC_{75}) based on the 3D viability studies. Next, we will assess the cell count for tumor growth. We will follow the same procedure as the previous tasks. We will assess 3D cancer invasion by introducing the 4 different concentrations of AMD3100 to inhibit CXCR4.

2.4 Combination therapy of AMD3100 and DOX: We plan to study whether inhibiting the CXCL12/CXCR4 pathway will sensitize the cancer cells to DOX. We will administer the drug based on the concentrations selected in 2.2 and 2.3. There will be a total of 16 combinations of DOX and AMD3100 concentrations (**Fig. 5**). For subsequent studies, we plan to investigate cell proliferation, viability, and invasion similar to the previous tasks (1.3). The results will be analyzed using the Chou-Talalay method[61,62] for drug synergy.

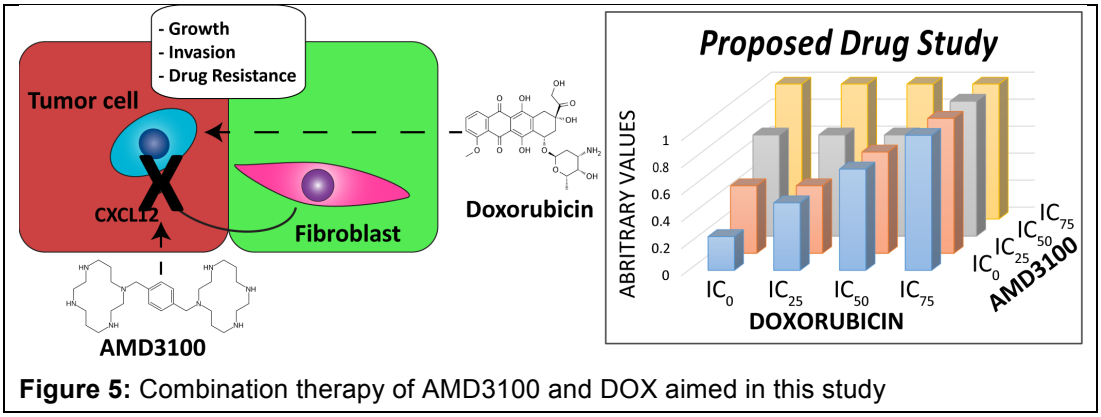


Figure 5: Combination therapy of AMD3100 and DOX aimed in this study

Expected Results and Contingency Plans: CXCL12/CXCR4 pathway has been shown to enhance cancer growth, invasiveness, and drug resistance within animal and conventional macroscale 3D hydrogels[20,39,54,60]. We expect that DOX will reduce viability and invasion of cancer cells in co-culture NFs as compared to CAFs. Furthermore, we expect that the addition of AMD3100 will block CXCR4 and enhance the effects of DOX. In the case that we do not see differences between days 1 and 3, we will extend the experimental time to 5 days.

Aim 3. To assess the role of fibroblast-induced desmoplasia in doxorubicin penetration and function.

Rationale: In addition to heterotypic signaling inducing anti-cancer drug resistance, there are biophysical factors[63] within the tumor microenvironment that can indeed inhibit the transport of drugs, thus limiting the efficacy of therapeutic regimens. Physical alterations with the stroma specifically include matrix deposition due to rapid proliferation of CAFs[4,14,42,64,65]. *In vivo* models of dense tumors require expensive microscopy, such as multiphoton laser-scanning microscopy and second harmonic generation[66,67], to observe the changes within stroma in real-time. In addition, it is difficult to analyze drug penetration within animal models due to spatial-

temporal complexities of 3D drug transport and a requirement to use imaging techniques such as MRI and CT[68]. Furthermore, transwell assays do not offer 3D complexities for co-culturing of cancer and fibroblasts to observe stromal remodeling[28,69]. Other microfluidic models have attempted co-culture of the cells but did not assess the effect of matrix remodeling on drug penetration[41]. Here, we will utilize our microengineered platform that can quantitatively analyze drug penetration with simple planar geometry. We will specifically focus on the deposition of matrix by fibroblasts and isolate the drug resistant effect due to the sole role of remodeled stroma.

3.1 Effect of cell-cell ratios on fibroblast-induced desmoplasia: Similar to task 1.1, we will introduce the cells (**Table 1**) into the system. At different time points (Day 0, 1, 3, and 5), the cell-embedded matrix will be fixed and stained for collagen-I (Alexa Fluor 555). Desmoplasia will be assessed by comparing the relative increase in collagen-stained areas across different time and conditions indicated in Table 1. Fiber morphology will be imaged using confocal reflectance microscopy[70,71] and quantified for fiber density and diameter.

3.2 Drug penetration and functional assessment in remodeled stroma: We will recapitulate the stromal remodeling by choosing the cell-cell ratio with the highest and lowest increase in collagen from task 3.1, and diffuse DOX (50 ng/mL) into the system to study drug penetration. For each time point (Day 0, 1, 3, and 5), the devices will be placed within our fluorescent microscope and imaged every 5 minutes for 30 minutes (**Fig. 1B**) up to 4 hrs. DOX will be excited at 543 nm and its emission will be collected at 590 nm[72]. The relative intensity profile of DOX from the channels to the tumor region will be quantified and compared between the 4 time points. Finally, viability and cell count after 24 hrs of DOX will be quantified to assess the drug function.

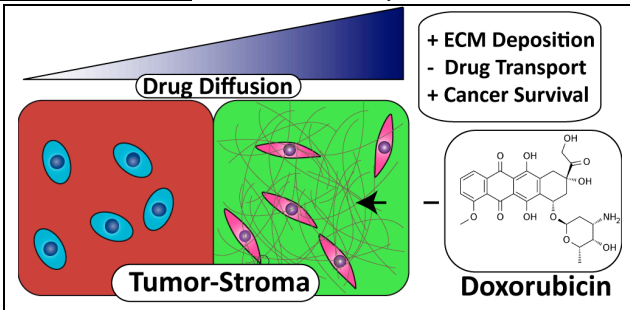


Figure 6: Schematic of drug transport through remodeled stroma

3.3 Stiffness quantification of fibroblast-induced desmoplasia: Fibroblasts (CAFs or NFs) will be encapsulated within collagen-I matrices in a 24-well plate. The cells will be cultured for different time points (Day 0, 1, 3, and 5). The stiffness change (Young's modulus) within the collagen matrices will be measured by atomic force microscopy (AFM) (MFP-3D AFM, Asylum Research) with silicon nitride tips (MSNL, Bruker) similar to our previous studies[73]. We will prepare cell-free collagen matrix at different concentrations (2mg/mL – 10 mg/mL) and perform AFM to correlate the stiffness to the fibroblast-induced changes.

3.4 Drug penetration and functional assessment in cell-free remodeled stroma: We will introduce cancer cells within the tumor region. However, collagen matrix of different concentrations will be loaded in the stroma to represent the remodeled stroma to isolate the biophysical alterations without cell-cell signaling. Drug penetration, viability, and cell count (consistent with 3.2) will be repeated to assess the function of the drug affected by stromal remodeling.

Expected Results and Contingency Plans: We expect that CAFs will deposit more collagen than NFs during the experiments. In addition, the increase in collagen density should reduce drug penetration to the tumor region subsequently reducing the effect of DOX. We expect that DOX will have reduced cell viability less so in highly dense collagen than one of lower density. If there is limited remodeling, we can extend the culture time to 7 days to allow for more ECM deposition.

E. MILESTONES

Aim 1: a) Form tumor-stroma consisting of cancer and fibroblast cells
b) Determine the influence of cell-cell ratios on cancer growth, viability, and invasion

Aim 2: a) Recapitulate the CXCL12/CXCR4 pathway
b) Assess and inhibit synergistic influence of fibroblasts on cancer invasion and drug resistance

Aim 3: a) Determine the influence of fibroblasts on stromal remodeling
b) Correlate increased ECM deposition to decreased drug transport and resulting drug function
c) De-couple biophysical cues from cell-cell signaling by utilizing cell-free remodeled stroma

Table 2: Proposed Timeline

Aims	6 mo	12 mo	18 mo	24 mo	30 mo	36 mo
Aim 1	→	→				
Aim 2		→	→	→		
Aim 3				→	→	→

Respective Contributions

During the first two years of time in the Nikkhah lab, I have made major headway with respect to this proposal. The first months since Fall 2014, I used to learn as much as I could about the field of research that is cancer biology, microfluidic devices used for cancer, and cancer invasion. I took this knowledge to design a microfluidic device that answered a gap within the field. During my research, I found that there were few *in vitro* platforms that tried to evaluate cancer migration in 3D that included a tumor and stroma components. I found several key articles stating the importance of how the stroma affects cancer invasion yet few models were developed to investigate this. Prof. Mehdi Nikkhah and I brainstormed and prototyped weekly until we finalized our design at the end of 2014. Then I fabricated the device and developed protocols for using it in 3D cancer invasion assays. We spent the next eight months on obtaining the preliminary data for this proposal by showing the capabilities of this device in understand 3D cancer invasion. During that time, we submitted one manuscript and three conference abstracts. The main research outcomes from that time was that we validated our model for cancer invasion in response to a chemoattractant, demonstrated high-resolution cancer invasion in real-time, showed 3D cell morphology using fluorescent microscopy. This research was only the first step before arriving at this proposal. We still needed to investigate how cells within the stroma affected 3D cancer invasion.

I (Danh Truong) wrote and developed the training proposal with guidance from the sponsor (Dr. Mehdi Nikkhah). We worked on brainstorming the initial ideas followed by the generation of the preliminary data. The co-sponsor (Prof. Joshua LaBaer) helped refine the hypothesis and the biological questions. Prof. Mehdi Nikkhah provided major inputs into the overall training proposal.

I (Danh Truong) will perform the research in this training proposal under the supervision of Prof. Mehdi Nikkhah. The sponsor and co-sponsor will be updated weekly and bi-weekly meetings will be held between the sponsors and the applicant.

Selection of Sponsor and Institution:

Arizona State University (ASU) is a “New American University” entailing comprehensive research that is economically, socially, and culturally responsible for the health of the surrounding communities. These values are relevant to me because of my commitment to developing affordable and live-saving bioengineered technologies to address healthcare challenges similar to my previous work in cardiovascular tissue engineering. ASU is well ranked in the top 10 intuitions in the U.S. for providing doctorates to Native Americans, Hispanic Americans, and Asian Americans. The university is a “knowledge enterprise” that caters to over 80,000 students of diverse backgrounds and of those students, over 13,000 are a part of the Ira A. Fulton Schools of Engineering. These undergraduate engineering students have the opportunity to participate in Fulton Undergraduate Research Initiative (FURI), which is a program that embraces young engineers by providing hands-on lab experience and independent research. Through the FURI program, I have interacted with several engineering (biomedical, mechanical, and chemical) undergraduates with excellent intellectual merit that are willing to conduct research independently. I myself have stepped up to the role of mentoring these undergraduates because it is important to me as a science educator to disseminate research knowledge and experience to the younger generations. I believe this institution offers an excellent environment for my passions in research and science education and aligns well with Kirschstein-NRSA predoctoral fellowship’ objective to enhance the diversity of the health-related research workforce.

My research training at my previous university, The University of Texas at Arlington, focused on the development of biomaterials for cardiovascular engineering and this influenced me to seek out well-known researchers that were involved in biomaterial synthesis. I accepted the invitation to the graduate recruitment weekend at ASU because of the outstanding biomaterials research activity of ASU. Upon my visit, I met Prof. Mehdi Nikkhah, an assistant professor in the School of Biological and Health Systems Engineering at ASU, who is an exceptional scientist with a well diverse background in biomaterials, microscale technologies involving cancer research and models, and tissue engineering. He received his extensive training at Harvard Medical School, Brigham and Women’s Hospital, Harvard-MIT Division of Health Sciences and Technology, and Virginia Tech. He explained to me how I could use my expertise to incorporate advanced biomaterials with microengineered technologies to create platforms that model diseased tissues. Inspired by his passion and his aggressive approach to develop translatable technology, I chose him as my mentor. Prof. Nikkhah provides students an independent working environment while giving guidance when needed. He has motivated me to develop my own protocols, learn grant writing, mentor students, and to pursue my project to the highest level of excellence. At this point, I have 2 publications and several conference abstracts in the Nikkhah Lab. In addition to the great research atmosphere, his laboratory is a diverse lab of students 18 students (i.e. 1 post-doc, 3 PhD, 5 MS, 8 Undergraduates, and 1 high school) from different backgrounds (i.e. Hispanic, Asian-American, Indian, Iranian, African-American) Not only do I experience a great amount of research, I have learned about different cultures that will help me with communicating science with others with cultures other than my own. I believe the training provided by Prof. Nikkhah will give me the foundation to become an excellent principal investigator that can develop and teach underrepresented students in science and engineering.

Alongside Prof. Nikkhah, I have selected Prof. Joshua Labaer, M.D., to co-sponsor me for my training. I met Prof. LaBaer when I signed up for his class, Biochemistry of Cancer, by the suggestion of Prof. Nikkhah. Prof. LaBaer is director of the Biodesign Instituate, an expert at discovering cancer biomarkers, and has developed his own technology (NAPPA) for discovering biomarkers that he has shared with the rest of the scientific community. He has a wide array of funding from agencies such as NIH, Mayo Clinic, Translational Genomics Research Institute, and Phoenix Children’s hospital, which suggests his expertise in cancer research, writing grants, etc. This will help me develop my own grant writing ability under his and Prof. Nikkhah’s guidance. Furthermore, Prof. LaBaer is on my PhD dissertation committee and is involved with my project. He has given me direction on how to develop my hypothesis and hone down on the scientific questions related to cancer. Additionally, he has successfully trained over 20 post-docs and 3 doctoral students from diverse cultural backgrounds. I believe his experience as a mentor and expertise in cancer biomarker will complement the training that I will receive from Prof. Nikkhah in biomaterials and microengineering platforms for development of tumor microenvironments model.

I have selected my sponsors and institution based on the following values: (1) school’s capacity to provide good research equipment and facilities, (2) availability and quality of professors that are willing to spend time with their mentees, (3) cultural diversity of the student population, (4) opportunities for working and teaching undergraduates, (5) enabling interdisciplinary research for people from all backgrounds as a knowledge enterprise.

Responsible Conduct of Research:

Arizona State University (ASU) is committed as a “New American University” requiring all students to conduct research responsibly. When I first joined ASU, I took an oath of responsible and ethical conduct of research along with my peers to uphold the highest standard in responsible research.

ASU and the National Science Foundation partnered up to lay out a plan for responsible conduct of research (RCR) training. There are three phases of training where the initial phase was to set up online courses in RCR training. The next phase was to set up seminars to educate the general student body and faculty members. Finally, the third phase was led by principal investigators to ensure high ethical standards in each individual research lab. I finished several modules already but plan to take more courses in the RCR online training to strengthen my knowledge in ethical practices and I intend to renew these courses every year. ASU has partnered with University of Miami to provide the online course modules at the Collaborative Institutional Training Initiative (CITI) website. The course modules encompass an all-around approach toward research integrity from conflicts of interest to research misconduct. From these modules, I have learned many new practices that I employ in my research today. Being more aware of the possible forms of misconducts, allows you to make better-informed unbiased decisions.

In addition to the course modules, ASU hosts seminars regularly that discuss bioethics and research misconduct. I recently went to one that talked about the bioethics of embryonic stem cells that also mentioned the research misconduct of the infamous STAP stem-cell study. At another seminar, we discussed the uses of CRISPR and the ethical precedence of designer genes and ownership of the CRISPR patents. Additionally, there are workshops that provide hands-on activities to learn more about research integrity and if you are unable to attend, these workshops are also recorded and placed online.

In our lab, Prof. Mehdi Nikkhah serves as a role model in performing significant research while maintaining ethical research practices. He has provided biosafety and ethical standards for the students in the lab to follow and he supervises all of the research. We work with animals regularly in our lab and have to follow the standard practices set up by the Institutional Animal Care and Use Committee (IACUC). Also, recently we have began to set up new protocols for obtaining human breast cancer tissues for extracting cells and extracellular matrices. We worked together with the Institutional Review Board (IRB) to review our protocols for ethical integrity. Furthermore, Prof. Joshua LaBaer as well as the other members of my thesis committee member provide excellent guidance in research professionalism and uphold me to high standards. I plan to commit to the highest level of research integrity and one day uphold my own students to such standards.

Goals for Fellowship Training and Career

Being a first generation Vietnamese-American, I never thought that I would become a researcher because a majority of my friends and family in the Vietnamese community were interested in health-related and engineering professions. As a child, I spent a lot of my time watching the television show, *Bill Nye the Science Guy*, and I believe that this has molded me into someone with a great passion for research and science education. However, I felt that I was trapped because I could not choose between healthcare and science. When I discovered the field of Biomedical Engineering, I believed it was the bridge that could connect my interests. However, as I learned more about the field, I realized I needed more training and knowledge. This drove me to pursue a Ph.D. level education because I wanted to expand my knowledge and use that newfound knowledge to engineer a tool to create a meaningful impact in healthcare.

My research at ASU is focused on developing microscale technologies to perform fundamental studies on cancer metastasis and therapeutics. Under the supervision of my sponsor and co-sponsor, Prof. Mehdi Nikkhah and Prof. Joshua Labaer, I have spent my first two years developing a novel three-dimensional (3D) tumor-stroma platform for the study of breast cancer invasion. My doctoral dissertation will consist of the development of this microfluidic device and its application in cancer research. My future training will consist of rigorous testing and validation of the newly developed platforms for co-culture and cancer drug studies, which I will perform in Prof. Nikkhah's laboratory. Already I have learned to fabricate microfluidic models, develop novel biomaterials for cardia tissue engineering, and study cancer invasion in different tumor models created in our lab. Additionally, I plan to work with my co-sponsor Prof. LaBaer, who is an expert in cancer biomarkers and personalized research. His lab is located on campus within 10 minutes of the Nikkhah Lab. There I will gain experience through multiple interactions with his group to learn about the biology of cancer and the kinds of questions that can be answered using our platform. I believe that this type of multidisciplinary approach of utilizing cancer biology and genetics together with biomedical engineering is what is needed to develop a comprehensive tumor model with high predictive power for clinical studies, personalized medicine, and biological findings. I expect that within the third year, I will accomplish the studies for Aim 1 and at least half of Aim 2. In the first half of the fourth year, I will finish Aim 2 and I plan to publish the results from Aim 1 and 2 in a peer-reviewed journal. Finally, I plan to spend the last 1.5 years of my doctoral studies on Aim 3.

In addition to research, I believe that science education is necessary for a cohesive community where everyone understands science at an equal level. I admire Bill Nye and Neil deGrasse Tyson for their ability to disseminate knowledge to the general public. To develop my career as a science educator, I plan to work on my public speaking and teaching abilities by instructing science and engineering classes as well as presenting my research at national conferences such as the annual meetings for Biomedical Engineering Society and American Association for Cancer Research. Additionally, my current university, ASU, offers a variety of avenues for interacting with undergraduates and the general public. I took the chance to lead tours in my laboratory and give talks about our research to high school and undergraduate students at recent university sponsored events. Furthermore, I have worked as a judge for senior research projects where I was given the chance to review and discuss other research areas with undergraduate seniors while also providing useful feedback for future projects. I plan on attending more research workshops and symposiums to expand my network for the exchanging of scientific knowledge as well as to learn new techniques that I can incorporate into my interdisciplinary research. I will also take any future opportunity to discuss and interact with others at symposiums by either being a judge or volunteering as an assistant. These opportunities will help me develop the necessary communication skills I need as a researcher and educator. Moreover, I am actively training and teaching undergraduates within my lab that are underrepresented (e.g. Hispanic, Filipino) in the scientific community. I have also had the privilege to attend a workshop for first generation graduate students. There, we discussed the difficulties of entering graduate school and I mentored first generation undergraduates who were interested in post-bachelors' education. I developed personal connections with these students and relayed my own experiences to them so that they can succeed in their own path.

Through my experiences, I learned that being the first of my family to enter college was difficult because I did not have a guide for the logistics of entering and completing college. Being Vietnamese-American and underrepresented in the scientific community made it difficult for me to transition to a Ph.D. level education. However, this will not stop me from achieving my career goal of becoming a principal investigator and guiding other Vietnamese-Americans to become active researchers in science and engineering. This fellowship will help me grow as one of the first few Vietnamese-American scientists in this field.

Activities Planned Under this Award

I plan to include a number of activities that will further develop my future professional career as an investigator in addition to research. During my first year at Arizona State University, I have already attended writing workshops, several seminars (outside of my research area), and meetings to promote research among minorities and undergraduates.

I believe that scientific communication is an important area of science that is severely lacking. Scientific illiteracy, such as not understanding the scientific method, are prevalent throughout the community. I aim to develop my ability to communicate and present science by not only attending conferences and seminars, but also by joining undergraduate clubs and mentoring students. As an example of my commitment, I have led tours and given talks to high school students about the research work that I am involved in and explained to them in a way they could understand it. I took full advantage of my time during these talks by interactively demonstrating my research and asking the students questions that will test their comprehension. Furthermore, my lab is partaking in programs that allows high school students to shadow our lab on a daily basis. Together, the high school student and I discussed current research in my field and we went over several scientific papers. I greatly enjoyed my time talking with the students and I will take any upcoming opportunities to give tours and talks to younger minds. I believe that for science to have a greater impact in society than it is necessary to help people be able to successfully understand scientists.

In order to develop my future career as a science educator and advisor, I have taken on several undergraduate students as mentees with diverse backgrounds from Indian, Filipino to Hispanic. Together, we spent time developing core laboratory skills (i.e. safety working in the lab, understanding and utilizing common lab equipment, advanced laboratory techniques, learning to microfabricate devices, biomaterials and cancer biology). I engaged my mentees by delegating tasks and testing them while still offering feedback and guidance when necessary. Watching them grow and learn has reminded me of when I was going under my own training in a previous laboratory. This experience has humbled me, and I believe that teaching is an important part of being a scientist because if I wasn't taught then these students would not have had the chance to learn and take part in science. This has led me to take my training and development very seriously.

ASU offers many programs that help with graduate career development and I plan to take part in two development workshops, Preparing Future Scholars (PFS) and Preparing Future Faculty (PFF). The former is a new interdisciplinary professional development that is offered as a one-year course to help graduate students enter industry. PFF is a nationally recognized program for doctoral students to pursue a faculty position or postdoc appointment after graduation. These programs will help me develop as a professional by offering me opportunities to be able to map out my career in a systematic way. After I finish these courses, I expect that I will have a clear understanding of the requirements and expectations of a faculty position or a career in industry.

I also plan to take part in more classroom studies to further my scientific knowledge. Prior to taking the course, Biochemistry of Cancer with Dr. Joshua Labaer, I knew very little about cancer biomarkers and intracellular pathways. Now I am able to formulate direct scientific hypotheses of cancer biomarkers' and pathways' role in cancer grown in a three-dimensional environment. This class was invaluable to me because it helped me focus my proposal toward greater scientific impact on cancer technologies. I plan to further study statistics, proteomics, and advanced genetic techniques to develop my toolbox as a researcher. I believe that the problems of today require a multidisciplinary approach to solve and that is why I became a biomedical engineer to utilize science and engineering to tackle healthcare issues such as the burden of cancer.

This funding will immensely help me develop and focus my career as a future investigator. I plan to fully take advantage of the fellowship by participating in educational and professional development resources (i.e. workshops, conferences, and training in labs at other universities) that I would have had to find funding elsewhere. I intend to not only focus on research but manage my time to develop myself to become a well-rounded scientist by disseminating scientific knowledge to the general public, mentoring undergraduate students, and learning new skills.

Doctoral Dissertation and Research Experience

My research experience began during my senior year of undergraduate at the University of Texas at Arlington in 2013. I participated in a dual-degree program where I studied over the course of five years to receive a dual degree in B.S. of Biology and M.S. of Biomedical Engineering. While taking the course Biomaterials with Prof. Yi Hong, I developed an interest in biomaterials and its use in current healthcare problems such as cardiovascular diseases. After class, I met Prof. Hong and asked to be a part of his lab to learn more about biomaterials and its application in cardiovascular tissue engineering. After joining, I worked together with a Ph.D. student and we developed an electrospun scaffold for small-diameter vascular graft. We produced a material that could reduce surface thrombosis and allow adequate blood flow. From this project, I developed skills in critical problem solving, scientific writing, data analysis techniques, and traditional bench work. Overall, we presented at three different conferences and published in the journal, *Acta Biomaterilia*.

After demonstrating that I was capable of working on my own, I began to learn how to synthesize polymers for cardiovascular tissue engineering. This helped me further develop my ability to carefully read and dissect scientific literature because I often had to look through articles to answer my questions on the protocols. We were able to successfully develop a novel material that I subsequently characterized for physical properties and effect on the viability and proliferation of endothelial cells. It was near this time that I graduated and transferred the project to another graduate student. Through this work, I laid the foundation for the future development of a novel polymer for cardiovascular repair. Overall, my research experience was invaluable because it taught me to seriously understand the rigorous work that is required for high quality research. Overall, I took full advantage of my time (1.5 yr) in the Hong Lab to gain expertise in working with biomaterials and tissue engineering. In the end, I was able to publish two journal papers and three conference abstracts.

MSc work, Journal Papers:

- Gao, G., Schilling, A. F., Hubbell, K., Yonezawa, T., **Truong, D.,** Hong, Y. & Cui, X. (2015). Improved properties of bone and cartilage tissue from 3D inkjet-bioprinted human mesenchymal stem cells by simultaneous deposition and photocrosslinking in PEG-GelMA. *Biotechnology letters*, 1-7.
- Punnakitikashem, P., **Truong, D.,** Menon, J. U., Nguyen, K. T., & Hong, Y. (2014). Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts. *Acta biomaterialia*, 10(11), 4618-4628.

MSc work, Conference Abstracts:

- "Sensory and Motor Enrichment using Molecular Guidance Cues," Neural Interface Conferences 2014, Dallas, TX, June 22-25, 2014
- "Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts," The Annual Celebration of Excellence by Students (**ACES**) symposium 2014, Arlington, TX, March 26, 2014
- "Electrospun biodegradable elastic polyurethane scaffolds with dipyridamole release for small diameter vascular grafts," 40 years of Bioengineering at UTA, Arlington, TX, March 19, 2014

After graduating, I entered Arizona State University in the summer of 2014 as a doctoral student and joined the lab of Prof. Mehdi Nikkhah. One of my recent achievements was fabricating a novel microfluidic chip for the study of breast cancer invasion in response to gradients of EGF. Before starting that project, Prof. Nikkhah came to me asked me if I could develop the protocols and expertise for microfluidics for our lab. I had to learn several new microfabrication techniques such as SU-8 photolithography and soft lithography. Once I created the microfluidic device, I worked on culturing cells within it. Later, I developed a microscope chamber to view 3D cancer invasion in real-time. The success of seeing cancer cells migrate allowed us to use our microfluidic device to modulate microenvironmental cues to control cancer invasion. We found epidermal growth factor enhanced cancer invasion within our platform similarly to animal models, which validated our

platform as a testing environment for cancer invasion. We prepared a manuscript based on our findings and have recently submitted it for publication.

Along with my work in microfluidics, I worked with other lab members utilizing my expertise to help complete the project. I co-led a project using my biomaterials expertise from my previous lab to create an injectable cell delivery system for cardiac repair. Together with the new material, we created an innovative method of characterizing cardiac cell beating behaviors *in vitro*. This project not only let me improve my research capacity but also my growth as a project lead. I had to coordinate with our lab members as well as with outside labs for experiments and data. In addition, I developed another tumor model with a graduate student to study cancer invasion using micropatterned hydrogels. I conveyed my experiences with my own project as well as helped design the experiments and write the manuscript. Both of these projects that I have mentioned were published last winter.

Lastly, in the Nikkhah lab, I have taken multiple opportunities to mentor undergraduate students. I spent the previous year mentoring a female student. Together, we built a physiological microscope incubator for our lab to perform real-time movies. We matured together as I became a better mentor and she became a better researcher. Recently, I have added a male Hispanic freshman and a female Indian Master's student to my team and together, we are working on manipulating the tumor microenvironment by modulating presence of stromal cells and inducing angiogenesis. After 1.5 years in this lab, I have worked on two published articles and one submitted manuscript and I also had the opportunity to present at numerous conferences.

PhD work, Journal Papers:

- **Truong, D.,** Puleo, J., Llave, A., Mouneimne, G., Nikkhah, M. Breast Cancer Cell Invasion into a Three Dimensional Tumor-Stroma Microenvironment. Submitted and Awaiting Revisions.
- Navaie, A.*, **Truong, D**.*, Heffernan, J., Cutts., J., Overstreet, D., Sirriani, R., Brafman, D., Vernon, B., Nikkhah, M. (2015) PNIPAAm-based Biohybrid Injectable Hydrogel for Cardiac Tissue Engineering . *Acta Biomaterialia*. *, Equal contributors
- Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M. (2016). A three dimensional micropatterned tumor model for breast cancer cell migration studies. *Biomaterials*, 81, 72-83.

PhD work, Conference Abstracts:

- **Truong, D**., Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," Molecular, Cellular, and Tissue Bioengineering Symposium, Tempe, AZ, April 1, 2016
- **Truong, D.,** Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M., "Three-dimensional (3D) Invasion of Breast Cancer Cells in a Well-Defined Tumor-Stroma Platform," ASME NanoEngineering for Medicine and Biology Conference, Houston, TX, February 20-24, 2016
- Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M., "A Three Dimensional Micropatterned Tumor Model to Study Breast Cancer Cell Invasion," Annual Biomedical Engineering Society (BMES) Meeting, Tampa, FL, October 7-10, 2015
- **Truong, D.,** Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," Annual Biomedical Engineering Society (BMES) Meeting, Tampa, FL, October 7-10, 2015
- Peela, N., Sam, S.F., Christenson, W., **Truong, D.**, Watson, W.A., Mouneimne, G., Ros, R., Nikkhah, M., "A Three Dimensional Micropatterned Tumor Model to Study Breast Cancer Cell Invasion," AZBIO Awards 2015, Phoenix, AZ, October 1, 2015
- **Truong, D.,** Llave, A., Puleo, J., Mouneimne, G., Nikkhah, M.," "Microengineered Breast Cancer Invasion Platform," AZBIO Awards 2015, Phoenix, AZ, October 1, 2015

1. Research Support Available

2. Sponsor's/Co-Sponsor's Previous Fellows/Trainees

Sponsor's Current Trainees
Dr. Nikkhah currently has 1 PostDoc, 3 Ph.D. graduate trainees and 3 MSc students. Mr. Truong is the first Ph.D. graduate student and joined in 2014. The other two joined in 2014 and 2015. In addition, there are 9 undergraduate students pursuing Honors thesis and independent research projects.

Previous Trainees:
Harpinder Saini, Master in BME, 2013-2015. Current Position: Ph.D. student in Nikkhah Laboratory. 4 joint publications.

Feba S. Sam, Master in BME, 2013-2015. 3 joint publications.

In addition Dr. Nikkhah has served on 5 PhD dissertation and 11 MSc thesis committees.

Co-Sponsor's Previous and Current Fellow/Trainees
Dr. Joshua LaBaer has trained 26 graduate students and postdoctoral fellows. Additionally, he currently has 8 graduate students.

Previous Trainess:

Bhupinder Bhullar, Post-Doctoral Fellow (2000-05) Current position: Scientist at Novartis, Lab Headat Novartis Institutes for Biomedical Research.

Pascal Braun, Graduate Student, Ph.D. (1997-03). Current position: Group Leader at Technische Universität München

Manuel Fuentes Garcia, Post-Doctoral Fellow (2004-08) Harvard Institute of Proteomics. Current position: Assistant Professor, Centro de Investigación del Cáncer/IBMCC, Departamento de Medicina y Servicio General de Citometría, University of Salamanca, Spain

Zahra Moradpour Post-Doctoral Fellow; (2008-10) Harvard Institute of Proteomics & V.G. Piper Center for Personalized Diagnostics, Arizona State University. Current Position: Department of Pharmaceutical Biotechnology, University of Medical Sciences, Shiraz, Iran

3. Training Plan, Environment, Research Facilities:
Danh is a student in the School of Biological and Health Systems Engineering at Arizona State University. He joined my lab in the summer of 2014 and plans to graduate by May 2018. Danh has completed his coursework in his previous Master's degree at the University of Texas at Arlington and plans to take his Comprehensive exam in the end of Spring 2016. His previous training consisted of core Biology, Biomaterials, and Tissue Engineering courses, which makes him well suited to pursue the proposed project. His thesis committee includes: Barbara Smith, Ph.D. (Assistant Professor, School of Biological and Health Systems Engineering), Joshua LaBaer, M.D.-Ph.D. (Professor of Chemistry and Biochemistry, Director, Virginia G. Piper Center for Personalized Diagnostics), Brent Vernon, Ph.D. (Associate Professor, School of Biological and Health Systems Engineering), and Ghassan Mouneimne, Ph.D. (Assistant Professor, College of Medicine, University of Arizona). This group will provide a broad array of interdisciplinary expertise in microfabrication, cancer cell biology, biomaterials and proteomics.

i. Training Plan
Nikkhah lab holds meetings once a week where the students are expected to provide short presentations on their research progress. In these meetings, Danh will discuss about his project, data, manuscript drafts, and future plans. Danh is also expected to review current literature and provide positive criticism and knowledge that he gained from the readings to other lab members. Additionally, Danh will work together with several

undergraduates that he mentors which will give him an opportunity to hone his skills in science education and mentoring. Danh is expected to meet with his committee members twice a year to provide updates to his project and gain insight and feedback from the members. He will be also expected to attend at least one major scientific meeting yearly where he will present his research in seminars and poster symposiums.

Danh's future as an investigator in cancer disease modeling requires a multidisciplinary training approach. We have set specific training goals that will provide the necessary skills he will need.

The goals are listed below:

1. Training in microfabrication and biomaterials
2. Training in cancer biology and invasion
3. Training in cell imaging and biostatistics
4. Training in leadership and educational outreach

Goal 1: Danh is an excellent researcher and has a deep interest in developing platforms for disease modeling by using his expertise in biomaterials and tissue engineering. His two previous publications demonstrated his expertise in developing biomaterials. Danh's long term interest is to model diseases like cancer in a microengineered platform and to incorporate biomaterials to produce physiological relevant models. Therefore, he will first need to develop his skills in microfabrication to be able to produce these platforms. Danh has already successfully completed courses in SU-8 photolithography, soft lithography, and created micropatterns onto silicon wafers. He will continue his studies by taking an elective in "Biomedical Microdevices," where he will expand his knowledge on BioMEMS and how to incorporate this into his research. The course will cover design and development of miniaturized systems for a wide range of biomedical applications from medical diagnostics to drug discovery and regenerative medicine. In addition to Danh's training in cancer invasion, he is also developing other disease models such as cancer angiogenesis and cardiovascular disease. Furthermore, Danh will receive training on how to isolate cells from patient-specific tissues. He will work directly with oncologists on this research. We intend to utilize these cells in our tumor models to create studies involving personalized diagnostics and medicine. Also, as one of his research goals, Danh intends to decellularize some of these tissues to create patient-specific ECM to introduce into the models. He has participated in several projects already that utilized his biomaterials expertise such as injectable hydrogel for cardiac repair, photopatterened hydrogel tumor model, and hydrogel microgroove topography for cardiac. His expertise in biomaterials and microfluidics will allow him to develop a niche to expand his own research in disease modeling in the future.

Goal 2: Danh's research proposal requires substantial training in cancer biology and specifically cytoskeleton organization, intracellular pathways, and therapeutics. His previous training as a Biologist during his undergraduate will help him understand and participate in cancer biology courses and discussion. We work closely with Prof. Ghassan Mouneimne, who is a cancer biologist (collaborator of Nikkhah lab) and is on Danh's committee, by sending weekly emails of research updates. Prof. Mouneimne provides excellent biological insights and questions that helps Danh to deepen his knowledge of cancer biology and its role in invasion. This exposure will help Danh refine his hypotheses and ability to generate novel observations and insights from his data. Porf. Joshua LaBaer, who is Danh's co-sponsor and is also on his committee, is a leader in personalized medicine and cancer biomarkers. The LaBaer lab investigates protein function *in vitro* by utilizing Nucleic Acid-Programmable Protein Array (NAPPA) in addition to high throughput studies *in vivo*.

Goal 3: Danh's research proposal requires detailed imaging and quantitative analysis of subcellular features. Our lab hosts a fluorescent microscope with 10x, 20x, and 40x objectives with 3D imaging capabilities. Additionally, the university has a Bioimaging Facility that houses multiple confocal microscopes capable of capturing 5 channels and imaging software that can perform multi-dimensional image processing in both xyz and xzy, FRET, FRAP, and lambda scanning. Our lab collaborator, Prof. Mouneimne, has expertise in imaging subcellular features in cancer cells and has readily available protocols that Danh can access. We recently had a field trip to the Mouneimne Lab, in Tucson, where Danh worked on analyzing real-time imaging of cancer invasion.

Goal 4: Danh's future as an investigator will require him to obtain good leadership skills in terms of management and mentorship. Our lab has a very good working environment and we try to hire students from

diverse backgrounds. Currently our lab hosts almost 20 members ranging from high school all the way to post doctoral fellows. Danh is expected to work together with graduate and undergraduate students by supervising and leading projects. He helps undergraduate students with their research goals as part of the Fulton Undergraduate Research Initiative (FURI). Danh is expected to guide their projects from finish to start. He has already helped complete three FURI projects and is working with three students on three more. Furthermore, our lab hosts educational outreach to local K-12 schools. Recently, we participated in a university wide outreach activity called Night of the Open Door. Parents and students from around the metroplex came to visit our lab and Danh was assigned as one of the tour guides. Lastly, Danh will be expected to teach courses and laboratory sessions.

ii. Environment

Arizona State University is an ideal environment for training in microengineered platforms for cancer disease modeling. The Nikkhah laboratory has extended expertise in translational research in producing microfabricated models for cancer and cardiovascular diseases. We have recently published several articles on hydrogel-based tissue engineering. Additionally, our lab has connections and interactions with hospitals (Mayo Clinic) and research institution (Translational Genomics) in the Phoenix Area. We work with the Mayoclinic to study patient-derived cancers. Also, there is a nano-fabrication facility on campus that provide a 100-class clean room for microfabrication. Next, there are excellent researchers at ASU that Danh interacts with daily. Dr. Barbara Smith, an expert in paper-based molecular diagnostics, olfactory sensing, and tissue imaging, shares lab space with the Nikkhah lab. She is a member of Danh's committee and is always available to discuss with Danh when he needs assistance. Finally, Dr. LaBaer will enhance Danh's training in cancer therapeutics as well as in general research with his decades as being a director at several high-level research facilities.

iii. Research Facilities

Nikkhah Laboratory: Dr. Mehdi Nikkhah has a well-equipped laboratory of four working benches and three dedicated rooms to microscopy, chemistry, and cell culture on the first floor of the Intergrated Technology and Science Building 1 at the Arizona State University Tempe Campus. The microscopy room contains a fluorescent microscope equipped with an apparatus for optical sectioning to image in three-dimensions. Additionally, there is a custom-built microscope incubator to be used for the live-imaging of cells. The lab has access to core facilities that provides autoclaves, dishwashers, PCR, western blots, confocal microscopy, SEM, and histology. I myself have my own desk and workbench that has access to statistical, image analysis, manuscript preparation, and graphical editing software. Additionally, there is a networked scanners, printers, and back-up storage.

LaBaer Laboratory: Before he was recruited to Arizona State University, he was the founder and director of the Harvard Institute of Proteomics. Prof. LaBaer is now the director of the Virginia G. Piper Center for Personalized Diagnostics and interim director of the Biodesign Institute. His lab is located in the basement of the Biodesign Institute. The lab features capabilities in NAPPA technology, epitope mapping, protein-protein interactions, high-throughput cell assays and cell cloning, sequence analysis, and protein purification. Additionally the lab has a complete collection of shRNAs that target human genes. Furthermore, Danh has connections with the students and research associates in the lab and is able to receive training and assistance in any of the technologies.

University: Arizona State University contains the W. M. Keck laboratories whichi house some of the latest bioimaging technology including, laser scanning confocal microscopy; video light microscopy; ratio-imaging fluorescence microscopy; atomic force microscopy; phosphorescence/ chemiluminescence/ radioisotope imaging; and computer-aided image processing. Additionally, the ASU NanoFab is a flexible nano-processing facility offerring state-of-the-art device processing and characterization tools which Danh has access and training to use.

4. Number of fellows/trainees to be supervised during the fellowship.

Danh Truong is one of three doctoral students in the Nikkhah Laboratory. There are three master's level students and two postdoctoral fellows.

5. Applicant's qualification and potential for a research career.

My acquaintance with Danh goes back to February 2014, when he was invited to ASU during graduate recruitment week. I have worked with many students who have great potentials in the field of Bioengineering. Based on this exposure to outstanding individuals, I believe that Danh was the finest and top student among other potential PhD candidates. His academic background, ideas, research findings, and his talent for developing and conducting research projects within an interdisciplinary framework, impressed me and prompted me to offer him a position, as a PhD student, in my group at ASU. Danh joined my group in August 2014 and started working in the areas of breast cancer metastasis, microscale system and biomaterials.

I am currently an Assistant Professor of Bioengineering at the School of Biological and Health Systems Engineering at ASU. Prior to ASU, I completed my Post-Doctoral Fellowship at Harvard Medical School and Brigham and Women's Hospital where I was also affiliated with Harvard-MIT Division of Health Sciences and Technology (HST). My research is centered on interdisciplinary areas related to the applications of micro- and nanoscale technologies in biology and medicine, tissue engineering, and cancer metastasis. I have published more than 30 journal articles and 50 peer-reviewed conference papers and hold five invention disclosures and one US patent. I have been also the recipient of numerous awards and recognitions during my career including: 2014 Fellow award from Cellular and Molecular Bioengineering Division of Biomedical Engineering Society, 2013 National Institutes of Health (NIH) Ruth L. Kirschstein National Research Service Awards for Individual Postdoctoral Fellows, 2011 "Outstanding PhD Dissertation in Engineering, Science and Mathematic" at Virginia Tech, 2010 "Graduate Man of the Year" at Virginia Tech, 2009 "Outstanding Doctoral Student in the College of Engineering" at Virginia Tech and so forth.

Since joining my group, Danh has developed a detailed research plan for his PhD dissertation and has proven to be a highly competent student. His current research is focused on the use of microengineering technology and advanced biomaterials to develop a biomimetic breast tumor microenvironment model on-a-chip. Despite significant progress in cancer therapy, there is a major gap in this field where researchers either use simple models like conventional plastic dishes or animal (i.e. rodent) models to perform drug screening. However, the tissue microenvironments in body are substantially different from plastic dishes or rodent models. In this regard, there needs to be better models, with native-like physiology, to study cancer. Danh's work is aimed to provide a versatile platform for physicians and researchers to perform fundamental studies on breast cancer metastasis in response to various microenvironmental cues and therapeutic compounds. We also envision that, this research will enable growing patient specific cancer cells to identify efficient therapeutic regimens, thus improving the quality of care.

Danh surprised me with the speed, quality and depth with which he managed both efforts and mastered the new research area. He has established himself as a hard-working and creative individual who strives for continuing accomplishments. However, what makes him an outstanding and exceptional student is his critical thinking, analytical problem solving, and inquisitiveness. In his first year of pre-doctoral work, he has already authored three highly reputable journal manuscripts two of which have been already accepted and pending minor revisions in the Journal of Biomaterials (Accepted, impact factor of 8.5) and Acta Biomaterialia (Pending minor revisions, impact factor of 6.0). He has also presented his work in two bioengineering related conferences. For his first project, there were several technical challenges that eventually, he had a creative breakthrough where he decided to revise the original design and create a new platform. In this process, he was able to successfully merge his unique background in cell biology and biomaterials, from his Bachelor's Degree in Biological Sciences and Master's Degree in Biomedical Engineering. We are currently collaborating with cancer biologists at the University of Arizona, in which Danh has demonstrated excellent communication skills and collaboration initiatives with them.

Danh himself is a Vietnamese-American and the first of his family to enter college as well as graduate school. Utilizing his cultural diversity, he has connected with other underrepresented students in science, technology, engineering, and math (STEM) and has recruited and mentored several of these undergraduate students in our lab. He has demonstrated strong work ethics and outstanding teamwork capabilities and proven himself as a highly respected colleague with valuable personal qualities and friendliness. He specifically understands that there is a greater need to bring cultural diversity to STEM. Thus, he has placed himself in a position to further

participate in the advancement of science for underrepresented students by joining and taking part in the Association for Women in Science and the Biomedical Engineering Society ASU chapter.

Our lab has had several opportunities to give talks to the scientific community and the general public. This past summer, high school students toured the campus and visited our lab. Danh was one of the key speakers in this tour who demonstrated excellent presentation skills with the talent to explain science to the students. Additionally, we went to biomedical engineering society conference in the beginning of October 2015, where he presented his work. He has aspirations to be in research as well as be a scientific educator. I have confidence that Danh will live up to his goals. Furthermore, he has a unique outlook, due to his Vietnamese heritage and circumstances growing up, which will bring cultural diversity to the biomedical engineering fields.

In summary, I truly believe that Danh is an exceptional graduate student with outstanding background. His expertise along with in-depth knowledge and skills in biomedical engineering and biology as well as leadership and excellent personal qualities will ensure his continuing success in the future. It is truly my pleasure to give Danh my ***highest recommendation*** for predoctoral fellowship in response to PA-14-148, titled “Ruth L. Kirschstein National Research Service Awards for Individual Predoctoral Fellowships to Promote Diversity in Health-Related Research (Parent F31 - Diversity).” I am absolutely confident that he will be a highly successful candidate for this award. This fellowship will immensely help him to achieve his academic dreams and goals as one of the few Vietnamese researchers in bioengineering. If I can be of any assistance, or provide you with further information, please do not hesitate to contact me.