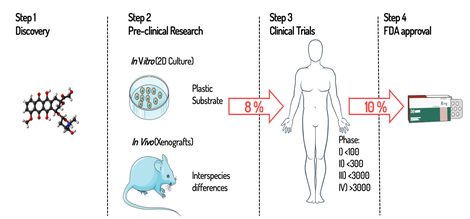
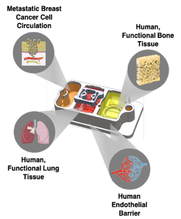
Recapitulation Of Organ-Specific Breast Cancer Metastasis Using An Engineered Multi-Tissue Platform

Alan Chramiec[1]*, Ece Öztürk[1,2], Miranda Wang[1], Kacey Ronaldson-Bouchard[1], Daniel Naveed Tavakol[1], Keith Yeager[1], Max Summers[1], Diogo Teles[1], Gordana Vunjak-Novakovic[1,3]
[1] Department of Biomedical Engineering, Columbia University, USA; [2] Research Center for Translational Medicine, Koç University, Turkey; [3] Department of Medicine, Columbia University, New York, NY, USA

Introduction

Although the 5-year survival rates for localized cancers are relatively high, those for metastases are <30% and have not improved significantly over the last 10 years, making metastasis the major cause of death from cancer. Traditional in vitro drug screening models are unable to faithfully recapitulate diseased human physiology, which, combined with the inherent weaknesses of xenograft-based in vivo preclinical models, leads to significant inefficiencies in the drug development process [figure below, left] (1,2). Organs-on-a-chip (OOC) try to more accurately mimic the topographical, electromechanical and biochemical cues in native human tissues, resulting in more faithful in vitro models. We believe there is a clear need for improved, dynamic, 3D, and human models of multi-tissue processes like metastasis in order to gain both a better understanding of the underlying mechanisms of it as well as improvements in treatments.. Breast cancer cell lines have been widely used to model metastasis within the tissue engineering field, including in published work from our lab. Furthermore, it is well known that breast cancer metastasis tends to have a subtype dependent organotropism, complete with unique molecular features at the various distal tissues. Here we show that using a novel organs-on-a-chip platform we have been able to recapitulate the multiple stages of both multi-tissue and tissue-specific breast cancer metastasis for the first time in an in vitro model [figure below, right].





Integrated Multi-Tissue Platform Development

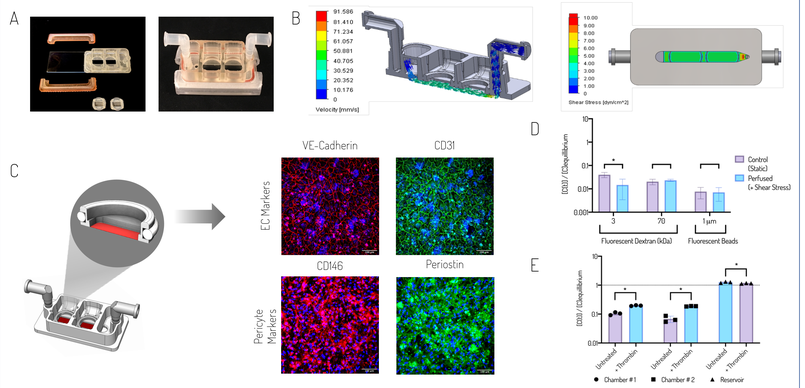


Figure 1: The multi-tissue platform [A] we designed allows us to co-culture bioengineered human bone and lung tissues. It features a channel connecting the tissues through which media and cells could circulate at physiologically relevant flow rates and shear stresses [B]. A selectively permeable vascular barrier featuring both mature endothelial cells and pericytes [C] is introduced to separate each individual tissue from the circulating media and metastatic cells. The function of these vascular tissues, as measured by resistance to the diffusion of fluorescent dextran and cell-sized beads, is maintained and in some cases improved as a result of exposure to perfusion-mediated forces [D]. Finally, vascular tissues are responsive to biochemical cues dictating their permeability [E].

Bioengineered Bone and Lung Tissues: Targets of Metastasis

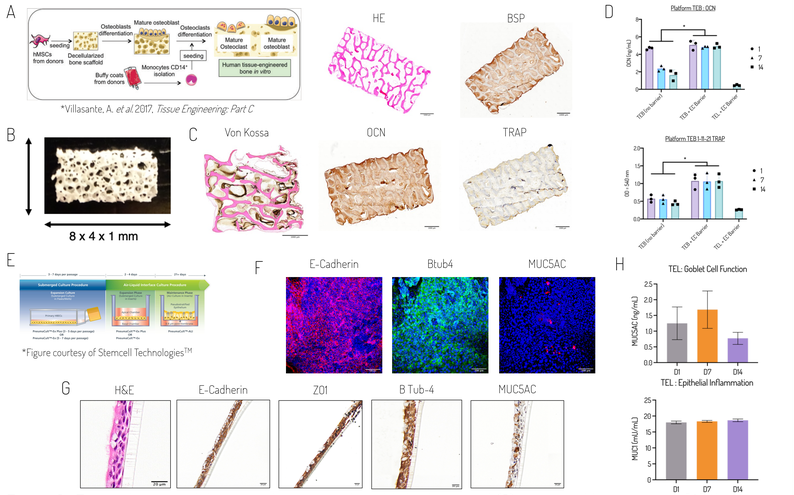


Figure 2: For our bone model, we used a previously published protocol (3) pioneered by our group [A] that uses a mineral bone scaffold [B] to enable the co-culture of mature osteoblasts and osteoclasts responsible for bone deposition and remodeling respectively. Their function, and it's subsequent maintenance within our integrated co-culture platform, was verified using IHC staining [C] and ELISAs [D]. Likewise, we used a previously established protocol [E] to create our bioengineered lung tissues, featuring mature human bronchial epithelial and goblet cells, as shown using IF [F] and IHC [G] staining, as well as with ELISAs [H] looking at MUCIN protein secretion.

Multi-Tissue Breast Cancer Metastasis

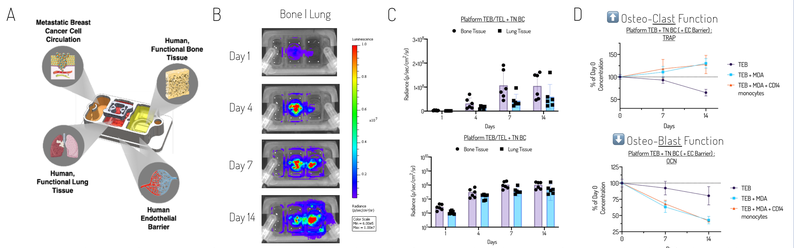


Figure 3: The combination of our organs-on-a-chip platform and inclusion of a vascular/endothelial barrier separating our individual bone and lung tissues from circulation was critical to not just the maintenance of tissue specific function, but also to the recapitulation of breast cancer metastasis across both tissue types [A]. Here you can see luminescence images [B] and their quantifications [C] monitoring the metastatic growth of luciferase-tagged MDA-MB-231 breast cancer cells at both bone and lung tissues within the platform. Additionally, we were able to recreate metastasis-specific processes well documented in breast cancer patients within this fully integrated system, including the multiple stages of the metastatic cascade and activation of the osteolytic bone cycle [D].

Organ-Specific Breast Cancer Metastasis

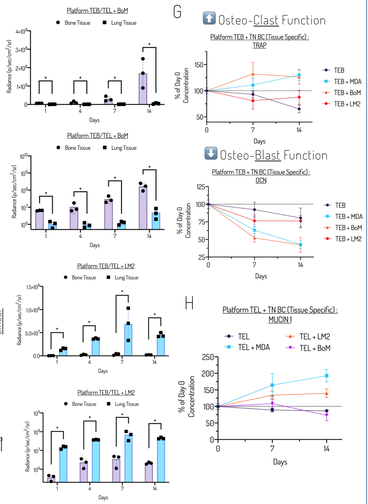


Figure 4: Finally, we used a modified version of our modular platform featuring both bone and lung tissues, but this time grown in isolation [A,B]. In combination with this setup, we circulated organ-specific breast cancer subclones of the original MDA-MB-231 parental line: the bone specific BoM-1833 line and the lung metastasizing LM2 line. This allowed us to recreate for the first time targeted bone and lung metastases previously only observed in mouse models. Both the bone- and lung-specific cell lines were luciferase-tagged, which meant that again we could use luminescence to visualize [C,D] and subsequently quantify [E,F] the metastatic growths of these cells. Just as in the small animal models, the bone targeting BoM-1833 cells exclusively metastasized and grew within our bioengineered bone tissues only [A,C,E]. Additionally, we found that just as with the parental MDA-MB-231 line highlighted in figure 3, these bone targeting clones also stimulated the osteolytic cycle in our bone target tissue, while any lung-targeting clones did not [G]. Conversely, the LM2 lung-specific line demonstrated preferential but not exclusive invasion of our lung target tissues, as compared to the bone-specific one [B,D,F]. Moreover, as with the parental MDA-MB231 line, metastasis of the lung-specific cancer cells triggered increased inflammatory-associated secretion of the cancer-marker MUCIN1.

Discussion

- ✓ Breast cancer metastasis is a complicated, dynamic, and clinically important process of cancer that impacts multiple organs and, based on the lack of progress with existing models, requires new ones to enable more impactful study.
- ✓ Our model system offers a variety of advantages: it is entirely human, yet manages to incorporate multiple tissue types, recreate multiple stages of metastatic progression, and capture the organ-specificity of various breast cancer subclones previously only achieved with small animal models.
- ✓ Moreover, metastases within our bioengineered tissues follow established organ-specific processes like the osteolytic cycle, and they allow us to pinpoint novel proteins involved in these growths.
- ✓ Going forward, we are performing scRNA-seq to identify both clones at the various stages of the metastatic process, and novel drug targets at metastatic sites across these multiple tissue types.

Contact

Alan Chramiec ac3904@cumc.Columbia.edu
Integrated in CMBS Graduate Program *COLUMBIA UNIVERSITY MEDICAL CENTER*
622 West 168th Street Department of Biomedical Engineering, VC12-234 New York, NY 10032

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