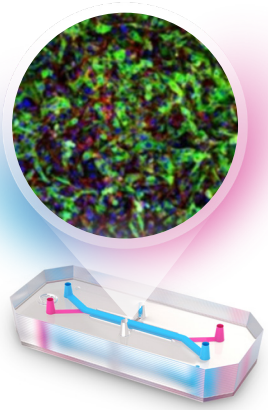
Creating Bone-Chip Models to Discover New Insights into the Mechanisms of Breast Cancer Metastasis



Introduction

Metastasis in breast cancer is a leading cause of mortality. In particular, metastasis of breast cancer to the bone significantly worsens patient outcomes, yet the mechanisms underlying this process remain poorly understood. While previous studies have focused on how interactions between cancer cells and bone marrow contribute to cancer metastasis, significantly fewer studies have been completed to understand how osteocytes—the primary regulators of the bone environment—influence metastatic breast cancer progression.

This case study summarizes how Dr. Stefaan Verbruggen and colleagues at Queen Mary University of London used Emulate Organ-Chips with the Human Emulation System to create a dynamic bone model of breast cancer metastasis, enabling them to elucidate the molecular mechanisms governing the interplay between cancer cells and osteocytes under conditions of mechanical loading.

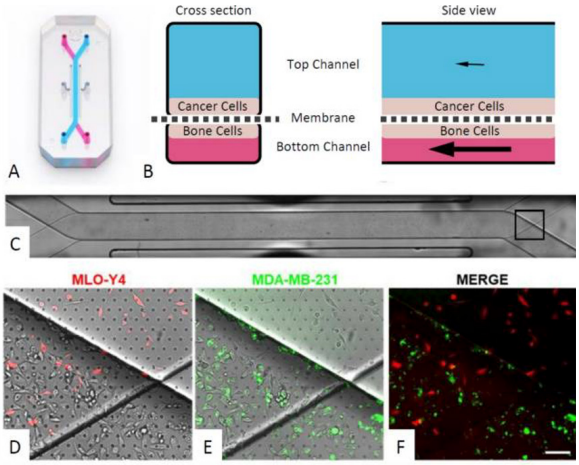


Figure 1. Schematic of a Bone-Chip model of early-stage breast cancer metastasis.

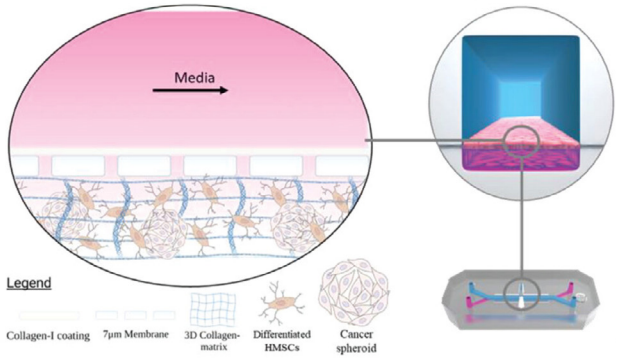


Figure 2. Schematic of an Organ-Chip model of late-stage human breast cancer metastasis.

Methods

Early-Stage Metastasis Organ-Chip Model

First, to understand the effects of mechanical stimulation on the early phases of cancer cell metastasis, MLO-Y4 bone cells (an osteocyte-like mouse cell line) and human breast cancer cells (MDA-MB-231) were seeded in an Emulate Chip-S1® Stretchable Chip (**Figure 1**). The bone cells (red) were seeded in the bottom channel, while the breast cancer cells (green) were seeded in the top channel, with the two channels separated by a porous membrane to enable crosstalk.

The standard fluid flow rate for both channels was set to 30 µL/hr. To generate mechanical loading, the flow rate in the osteocyte channel was increased to 1,000 µL/hr, which generated a shear stress of 0.03 Pa.

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Late-Stage Metastasis Organ-Chip Model

Next, to develop a fully human model of established metastasis, the researchers used osteogenically differentiated human MSCs and cancer spheroids (**Figure 2**). These were combined with a collagen solution and seeded into the bottom channel of the Chip-S1. Flow was applied to only the top channel at a rate of 30 µL/hour.

Results

Organ-Chip model reveals that mechanical loading of osteocytes leads to increased cancer cell invasion

To interrogate the effects of mechanical stimulation of osteocytes on breast cancer metastasis, Dr. Verbruggen created an Organ-Chip model of breast cancer bone metastasis[1]. As shown in **Figure 3**, the model features breast cancer cells (EpCAM, green) in the top channel and osteocytes (identified by the absence of EpCAM) in the bottom channel. Images in the upper panel are from Organ-Chips that were exposed to minimal shear stress, while images in the lower panel are from Organ-Chips that were exposed to high shear stress. As can be seen in both the orthogonal view and the magnified region of the osteocyte channel, applying shear stress increased the invasion of cancer cells from the top channel (breast cancer compartment) to the bottom channel (osteocyte compartment).

Mechanical stimulation of osteocytes decreases migration of cancer cells

To elucidate how mechanically loaded osteocytes regulate breast cancer cell migration, breast cancer cells stained with CellTracker Green were seeded into the top channel of the Chip-S1 consumable and cultured for 8 days alongside osteocytes in the bottom channel under the same mechanical stimulation conditions as previous experiments. Migration of breast cancer cells with and without shear stress was determined by imaging the chips over the course of 12 hours and using a standard cell migration tracking plugin from ImageJ. As shown in **Figure 4**, when osteocytes were exposed to shear stress, breast cancer cells exhibited significantly less migration.

Future Directions: Using Organ-Chips to understand metastasized tumor progression in bone

Throughout his work, Dr. Verbruggen has shown that metastasized tumor growth is a balance between the amount of TNF-α secreted by osteocytes and the amount of TGF-β secreted by cancer cells[2].

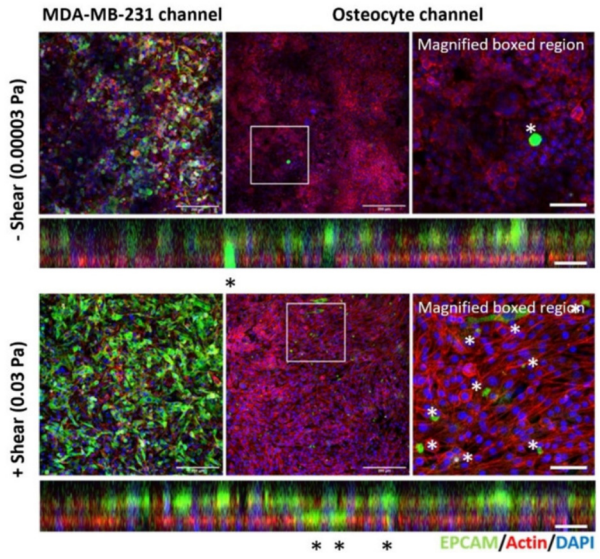

Figure 3. Effect of mechanically loaded osteocytes on cancer cell invasion. *Immunofluorescent staining for cancer cells (EpCAM, green), actin (Phalloidin, red), and nuclei (DAPI, blue) in both the cancer cell channel and the osteocyte channel. Invasion of EpCAM-stained cancer cells through the porous membrane into the bone channel are indicated (*). Scale bar = 20 µm.*

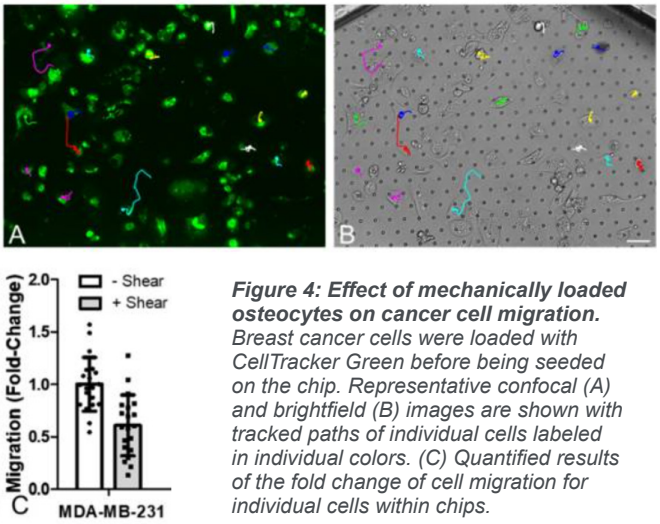

Figure 4: Effect of mechanically loaded osteocytes on cancer cell migration. *Breast cancer cells were loaded with CellTracker Green before being seeded on the chip. Representative confocal (A) and brightfield (B) images are shown with tracked paths of individual cells labeled in individual colors. (C) Quantified results of the fold change of cell migration for individual cells within chips.*

Dr. Verbruggen's model of metastatic tumor progression suggests that, in the early phases of metastasis, tumor growth is slowed by TNF-α secretion. However, once a critical tumor size is reached, the cancer cells secrete enough TGF-β to overcome this suppressive effect, leading to more rapid tumor growth. This process has been shown to be dependent on a primary cilium-mediated mechanism, demonstrating once again that mechanical forces play a critical role in regulating the metastatic progression of breast cancer within the bone.

The next steps within Dr. Verbruggen's research program are to better understand these mechanisms of tumor progression during the later stages of metastasis and determine how they are influenced by mechanical forces. To accomplish this, Dr. Verbruggen has developed a model of established tumor metastasis that will enable him to interrogate these mechanisms. In this model, human cancer spheroids are suspended within a hydrogel and co-cultured alongside osteogenically differentiated human MSCs (**Figure 5**) in the bottom channel of Chip-S1. Media flow in the top channel provides mechanical stimulation and mimics the native tumor microenvironment of an established *in vivo* breast cancer metastasis within the bone.

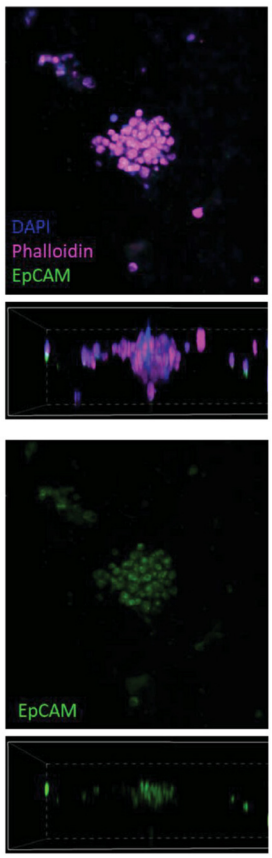

Figure 5. Organ-Chip model of established breast cancer bone metastasis. *Human breast cancer spheroids (green) were cultured alongside hMSCs. The cytoskeleton (Phalloidin, pink) and nuclei (DAPI, blue) of both cancer and osteocyte cells are shown. hMSCs can be identified by the lack of EpCAM expression.*

Discussion: How Organ-Chips Benefit Cancer Research

2D monolayer cultures are widely recognized to have limitations in cancer research due to their lack of representation of the tumor microenvironment, which is particularly challenging for researchers trying to understand endothelial to mesenchymal transition (EMT) mechanisms. While animal models provide more biological complexity, they suffer from species translation issues and an inaccessible tumor microenvironment, limiting their utility in elucidating the mechanisms underlying human cancer progression. Organ-Chips offer a unique modality for obtaining human-relevant cancer insights through a number of features:

How Organ-Chips Benefit Cancer Research

Tissue co-culture:

The two compartments of an Organ-Chip enable researchers to create co-culture models of distinct tissue types. The porous membrane separating the two channels enables crosstalk between cells as well as cell migration from one compartment to another, thus allowing researchers to quantify the extent of cancer cell migration and invasion.

Selective application of shear stress:

Zoë® Culture Module enables researchers to apply selective shear stress by individually controlling fluid flow rates within each channel. This precise control allows researchers to stimulate only one cell population in order to quantify the effects of shear stress on their tissue model.

Complex 3D tumor microenvironment:

The architecture of an Organ-Chip enables researchers to more accurately replicate the complexity of the tumor microenvironment by modeling a three-dimensional tissue that can incorporate elements such as extracellular matrices, tumor spheroids and organoids, and immune cells alongside physiologically relevant biomechanical forces such as stretch and shear stress.

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Traditionally, studying the effects of mechanical loading on osteocyte regulation of cancer cells in conventional 2D cell culture models requires culturing osteocytes on a rocker for a length of time to stimulate the release of cytokines and then transferring the conditioned media to a separate flask of cancer cells. In this study, the Organ-Chip model of breast cancer bone metastasis demonstrated that bone and cancer cells interact very differently under static versus mechanically loaded conditions. Importantly, this study found key differences in osteocyte regulation from previous studies that had been conducted in 2D cell culture.

As shown in **Table 1**, some key differences have been observed between the two experimental methods. Conditioned media from osteocytes that received mechanical loading decreased the migration and invasion of breast cancer cells, but stimulated their proliferation. Organ-Chip experiments showed similar results for migration regulation, but did not demonstrate that mechanical loading influences proliferation. Interestingly, Organ-Chip experiments showed that shear stress increases breast cancer cell invasion, which is the opposite effect of what conventional 2D cell culture models show. These discrepancies between 2D cell culture and Organ-Chip experiments highlight the importance of incorporating more complex and physiologically relevant *in vitro* models such as Organ-Chips into cancer research studies to better elucidate underlying molecular mechanisms of cancer progression.

Breast cancer cell behavior	2D Culture	Organ-Chips
Migration	↓	↓
Proliferation	↑	↔
Invasion	↓	↑

Table 1: Effect of fluid shear on osteocyte regulation of cancer cells across in vitro models.

Conclusion

With the help of the microfluidic Organ-Chip model, Dr. Verbruggen and colleagues demonstrated the importance of replicating the mechanical tumor environment, which enabled them to show that mechanical stimulation of osteocytes leads to increased invasion potential of cancer cells. Understanding this mechanism will help the research team develop new treatments for breast cancer bone metastasis, which currently has very poor survival rates.

Reference Publications

1. Mechanical Stimulation Modulates Osteocyte Regulation of Cancer Cell Phenotype.
2. A Novel Primary Cilium-Mediated Mechanism Through which Osteocytes Regulate Metastatic Behavior of Both Breast and Prostate Cancer Cells

Comment From the Researcher

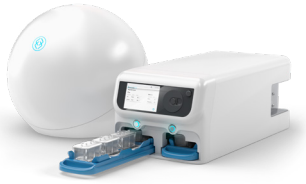
"The field of tumour microenvironment research is likely to benefit from the expanding use of Organ-Chip models such as the Emulate platform. In our studies, the Human Emulation System enabled precisely controlled levels of fluid shear stress to be selectively applied to bone cells within a microfluidic channel. At the same time, we were able to monitor cancer cell behaviour in a separate channel that was connected to the bone cells by a porous membrane. In so doing, the Organ-Chips created a more realistic tumour microenvironment that enabled us to better understand how bone cells regulate cancer cell behaviour and metastasis."

Stefaan Verbruggen, PhD
Queen Mary University of London

Related Products

Human Emulation System

The Human Emulation System is a complete Organ-on-a-Chip solution that enables unprecedented insights into human biology across a variety of organ models. At the heart of the Human Emulation System is Zoë-CM2™ Culture Module, which automates the precise conditions needed to culture up to 12 chips.



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