

SINERGIA

Microfluidic platform to personalize the therapy of bone tumors: a focus on breast cancer and lymphoma





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INTRODUCTION

Bone metastasis are notoriously hard to treat and tend to survive treatment due to drug resistance caused by the bone microenvironment [1,2]. Here, we focus on breast cancer and (NHL), which are known for non-hodgking lymphoma metastating to bone.

Currrent drug-testing models are not complex enough to accurately recapitulate the bone biology and architecture.

Based on previous models designed in our lab [3], this project focuses on the biofabrication of 3D human bone model for drug screening with two structures of different stiffness (high-stiffness ion-coated structure cells differentiation and low-stiffness bone tor hydrogel for vessel formation)

METHODS

Non-Hodgkin lymphoma

Simplified version of the model

Day 1

Seeding of NHL cells with and without the bone marrow microenvironment EGM-2-MV

NHL (0.8 M/mL) BMSC (0.6 M/mL) and/or HUVECs (3M/mL)

Day 3

Addition of FDA-approved drugs at different concentrations Venetoclax: 100nM to 10 000nM <u>VL51</u>: Copanlisib: 1nM to 500nM Rec1 and TMD8: Ibrutinib: 0.1nM to 10 000nM

Day 6

High-throughput screening of dose-dependent mortality with highcontent screening microscope (ImageXpress microscope HCA)

Breast cancer

Addition of osteoclasts and osteoblasts

Week 1

Seeding of primary monocytes on DLP 3Dprinted, SBF-coated resin insert Diferentiation of monocytes into osteoclasts

Week 3 – day 1

Seeding of primary BMSC-derived osteoblasts on the insert

Week 3 – day 2

Seeding of primary HUVECs, primary BMSCs and breast cancer cells in a hydrogel at the center of the insert Insert placed in Ibidi





SBF = simulated body fluid BMSCs = bone mesenchymal stromal cells HUVECs = human umbilical vascular endothelial cells



Cancer cell proliferation & drug resistance - NHL

Fibrin gel -



Microenvironment-mediated drug resistance



- Increased cell mortality with drug concentration when 2D cultured cell lines were exposed to an anti-NHL drug.
- Co-culturing NHL cells with BMSCs and endothelial cells in a 3D microenvironment seemed to exert protective effects.
- Results in agreement with xenograft models demonstrating that the tumor volume increases over time when mice are treated with any of these drugs due to the onset of drug resistance.
- Increase of multiple cytokines' expression in coculture

Endosteal niche cell activity



Endosteal niche shows sign of proper tissue maturation and expression of bone activity markers: Osteonectin (OSN) for OBs (left) and TRAP for OCs (right)

Perivascular niche organization



HUVECs + BMSCs + MDA in fibrin gel day4 post-seeding

compared to monoculture (*i.e.* 1L6, 1L8, chitinase 3–like 1, serpin E1) Overall, the biofabricated 3D microenvironment more closely mimics the *in vivo* response of NHL cells to drugs.

In co-culture of HUVECs and BMSCs, presence of vascular network throughout the whole gel + presence of lumen in the vessels

CONCLUSIONS

This bone-on-a-chip is able to develop a fully-formed vascular network in less than 4 days and shows the preservation of differentiation markers of bone cells. It also allows for the clustering and proliferation of cancer cells (i.e. breast cancer and NHL cells), as well as highlights the existence of microenvironment-mediated drug resistance.



This model could be used for drug screening or personalized medicine. It would allow to predict which compound, dose and combination would suit each patient best. This would improve the patient's quality of life by enhancing the chance of sensitivity to the drugs as well as reducing side effects.

[1] K. Venetis et al., 'Breast Cancer with Bone Metastasis: Molecular Insights and Clinical Management', Cells, 2021

[2] J. O. Armitage et *al.*, 'Non-Hodgkin lymphoma', The Lancet, 2017

[3] M. V. Colombo et al., 'Engineering the early bone metastatic niche through human vascularized immuno bone minitissues', Biofabrication, 2021

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