Poster #: 1207

XXI IwCLL International Workshop on CLL

In vitro investigation of the phenotype of CLL cells during trafficking and homing in a 3D-printed bioreactor

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OBJECTIVES

- Establish a new model for investigating ex vivo trafficking and extravasation of CLL cells:
  - Develop a 3D bioprinted model selectively perfused by a 3D printed bioreactor (VesselBox)
  - Ensure the biocompatibility of VesselBox with cellular viability
  - Assess scaffold maturation and functionality
- Characterize CLL immunophenotypic changes induced by tissue-specific microenvironment
- Evaluate chemokine- and drug-mediated mobilization (CCL19, BTK inhibitors)

CONCLUSIONS

- VesselBox enables the study of CLL dissemination, dynamic phenotype, and response to therapies
  - Allows the identification and characterization of specific CLL subset (CXCR4high/CD5high, linked to tissue homing and proliferation) (1,2)
  - Reproduces drug-induced mobilization mimicking in vivo effects
- Adaptable for the study of other malignancies by customizing scaffold composition
- Provides a translational platform bridging static in vitro systems and in vivo models

Scan the QR code for additional 3D reconstruction of a 3D bioprinted CLL microenvironment

INTRODUCTION

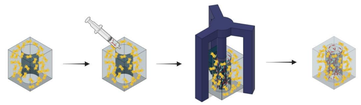
- Trafficking of malignant B cells between peripheral blood, bone marrow, and lymph nodes is crucial for CLL survival, proliferation, and therapy resistance
- Current in vitro models fail to reproduce dynamic trafficking and extravasation (3)
- 3D bioprinting enables fabrication of multi-material and multi-cellular three-dimensional scaffolds increasing the reliability and physiological relevance of in vitro models. Previous 3D bioprinted CLL models support long-term viability (up to 28 days), allowing in-depth investigation of the biological and molecular mechanisms underlying the disease (4)

METHODS

1. Bioreactor design and 3D printing

VesselBox includes: I. a base II. a homing module to allocate the scaffold III. a lid with optical window IV. two adapters to selectively perfuse hollow scaffolds in a sterile and sealed environment. The VesselBox is 3D printed with autoclavable materials.

2. 3D bioprinting and casting fabrication of a vascularized lymphoid microenvironment



Firstly, Human Lymphatic Fibroblasts (HLF) are bioprinted in a hollowed scaffold, that is then filled by manual casting with a mixture of endothelial and mesenchymal stem cells; a 3D printed plastic rod is inserted in the middle before photocrosslinking and the hollow channel is generated after gently removing the rod.

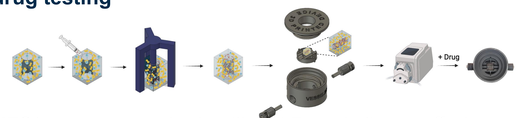
3. Vascular barrier maturation assessment

IF for endothelial markers (CD31, Ve-Cadherin, VWF) in confocal microscopy

4. Vascularized scaffold perfusion and leukemic cells dissemination monitoring

- Scaffold is perfused using a peristaltic pump connected to the VesselBox device
- MEC1-GFP/Primary CLL cells (n=3) are circulated, sampled at 1, 3, and 7 days
- Extravasation is assessed by IF, immunophenotypic changes by FC, gene expression by RT-qPCR

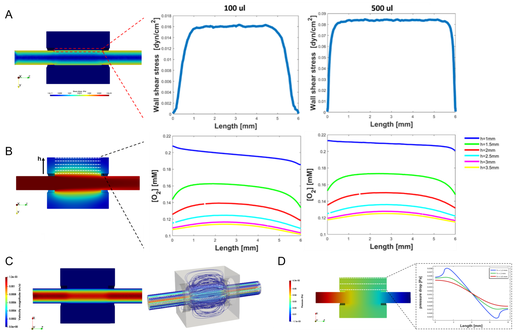
5. Biofabrication of a leukemic microenvironment and drug testing



MEC1 cells are mixed with HLF and the scaffolds are biofabricated as shown above. Drugs can be added to circulating medium and their effect on leukemic cells can be assessed.

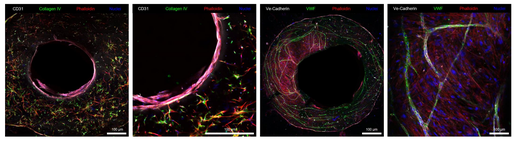
RESULTS

1. CFD simulations confirm the full compatibility of the VesselBox with dynamic cell cultures



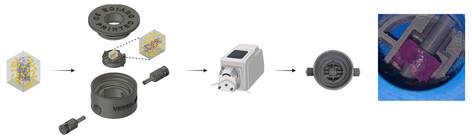
Computed pressure gradients (1A), flow distribution (1B), oxygen concentration (1C) and shear stress (1D) within perfused scaffolds are compatible with embedded cells viability.

2. Endothelial cells form a vascular network



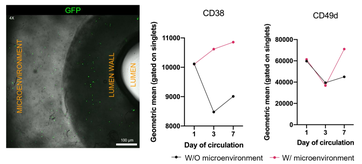
14 days of static maturation are required to endothelial cells to form a compact layer in the middle and a vascular network that can be assessed by endothelial markers (CD31, Ve-Cadherin, VWF)

3. VesselBox allows the selective perfusion of the vascularized scaffold



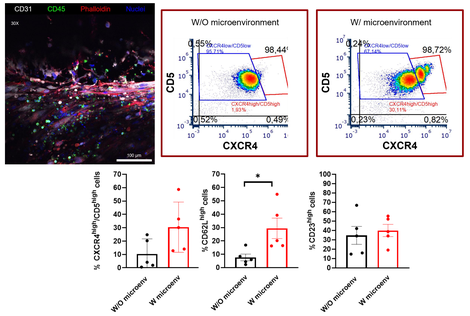
After 14 days of static maturation, the vascularized scaffolds are perfused in the VesselBox device connected to a peristaltic pump. The adapters ensure that medium only passes through the inner hole of the scaffold, guaranteeing a selective perfusion.

4. Circulating MEC1-GFP cells disseminate in microenvironment laden vascularized scaffolds



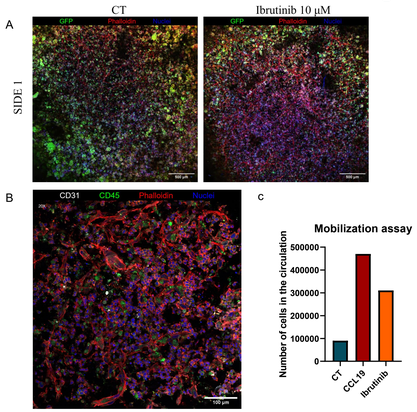
Circulating MEC1-GFP cells cross the endothelial barrier, disseminating towards the vascular and microenvironment compartments 3 days post-perfusion. Upregulation of CD38 and CD49d suggest a cross-communication between CLL and microenvironment cells.

5. Circulating primary cells interact with endothelial barrier and modulate tissue-homing markers



Primary cells circulating for up to 7 days within the VesselBox system are able to interact with the endothelial layer, mirroring the trafficking between blood and secondary lymphoid tissues. Moreover, the upregulation of CXCR4, CD62L, CD23 indicate a tissue-homing and active phenotype of CLL cells.

6. 3D Bioprinting allows the establishment of a model that includes MEC1 cells suitable for drug testing



MEC1 and HLF are bioprinted together. After preliminary studies demonstrate that ibrutinib reduces the number of MEC1 cells and their clusters in the scaffold (6A), mobilizing effect of ibrutinib is tested in the complex vascularized tissue. MEC1 cells are bioprinted with HLF in the microenvironment. The two populations homogenously populate the scaffold after 14 days of static maturation and up to 5 days of dynamic culture in the VesselBox (6B). After 5 days of circulation in presence of CCL19 and ibrutinib, mobilization from the microenvironment towards circulation is observed (6C).

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ACKNOWLEDGMENTS

C.S. acknowledges financial support from EHA Advanced Research Grant 2020. Associazione Italiana per la Ricerca sul Cancro AIRC under IG 2018 - ID. 21332 and Special Program on Metastatic Disease – 5 per mille #2119. We acknowledge Alembic and Fractal facilities.

DISCLOSURES

The authors have no conflict of interest to disclose.