Metastasis on-a-chip platform to follow in real-time the extravasation process and collect extravasated cancer cells

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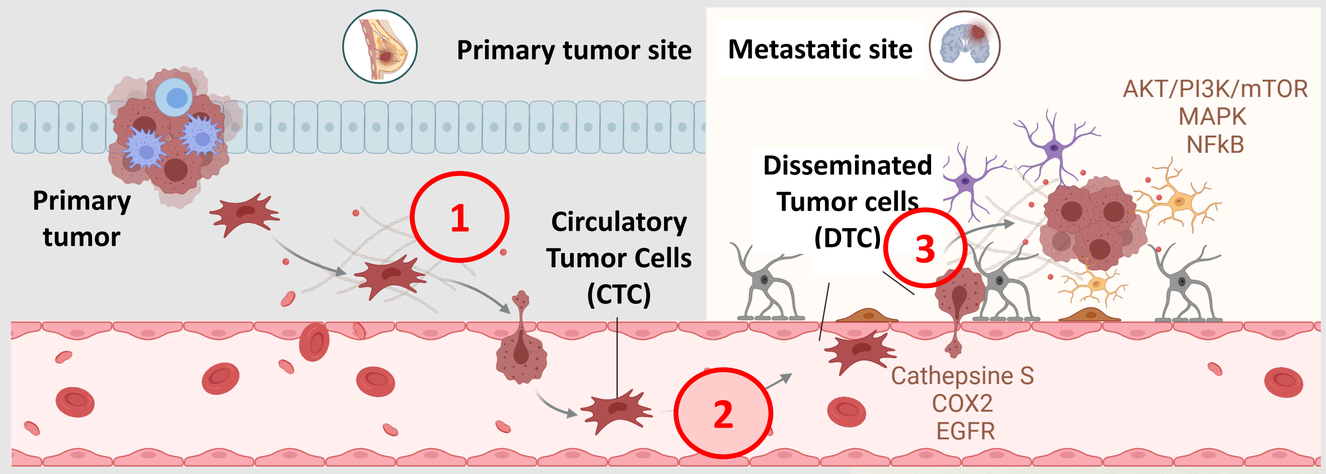
Background: Metastasis in breast cancer

- Most diagnosed cancer in the world

- **90%** of mortality in breast cancer is due to the formation of metastasis

Cancer cells Extravasation: A Highly Dynamic Process



1. Intravasation: Detachment from the primary tumor and entrance in blood circulation

2. Distribution: Flowing inside blood circulation to distant targeted organs

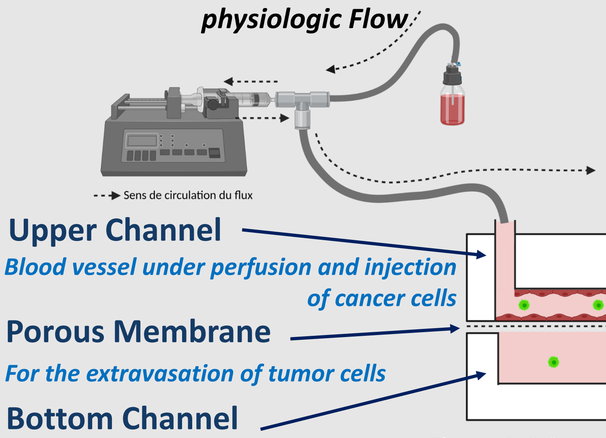
3. Extravasation: Migration trough the endothelium wall and exit from blood vessels

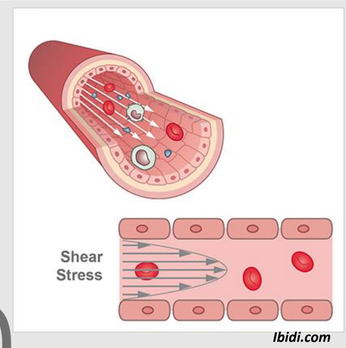
Aim of this study : Developp a metastasis on-a-chip platform to follow, collect and caracterise tumor cells that are able to extravasate

Metastasis on a chip device and microfluidic platform

Microfluidic Pump

To perfuse liquid inside the microdevice and apply a physiologic Flow





Shear stress in blood vessel

$$\tau = \frac{6.\mu.Q}{w^2.h}$$

τ is the wall shear stress in dynes/cm², μ is the viscosity of medium (μ=0. 0078 s.dynes/cm2 for standard culture medium), Q is the flow rate (m³/s), w the width (m)and h the height (m) of the channel

***Figure 1.* Schematic** Side view of the metastasis on a chip microdevice

3D Blood vessel in the Metastasis on-a-chip device

Upper Channel: Blood vessel under perfusion

Photo of the metastasis on a chip device

Bottom Channel: to collect extravasated cancer cells

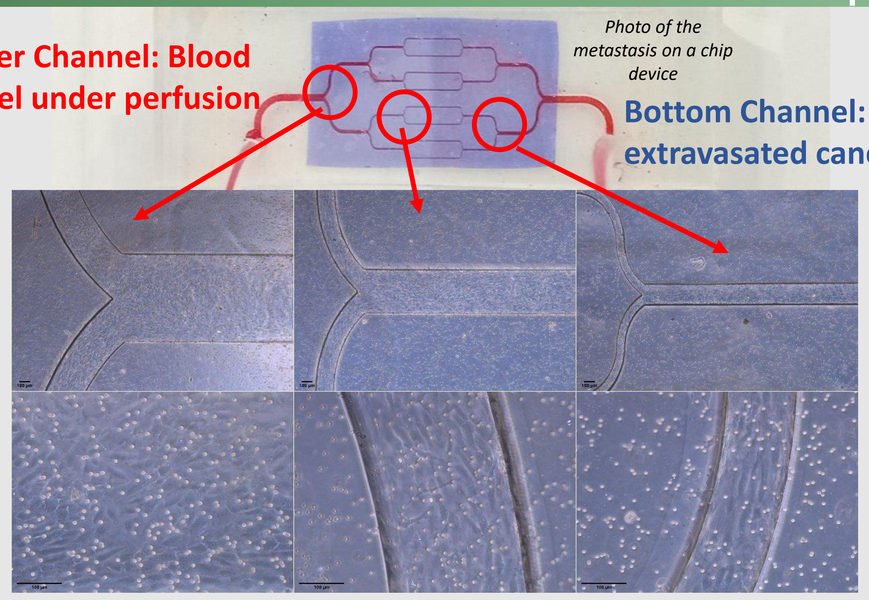


Figure 2. HUVEC cells inside the device after 4 days of dynamic culture conditions. From left to right inside a 800 µm, 400 µm and 200 µm channels width. All the channels have a 800 µm high. Up, 5X magnification objective. Down, 20X magnification objective, DmI1 Leica Microscope

Complete 3D endothelium

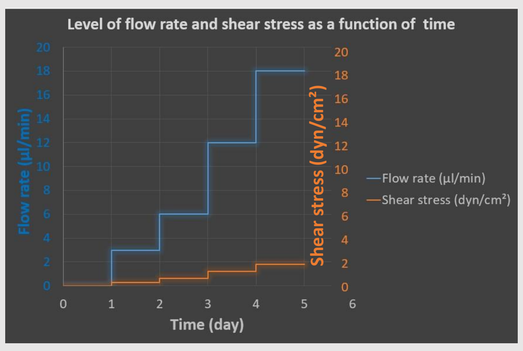


Figure 3. Flow rate and shear stress as a function of time, from day 0 to day 5 of dynamic culture conditions

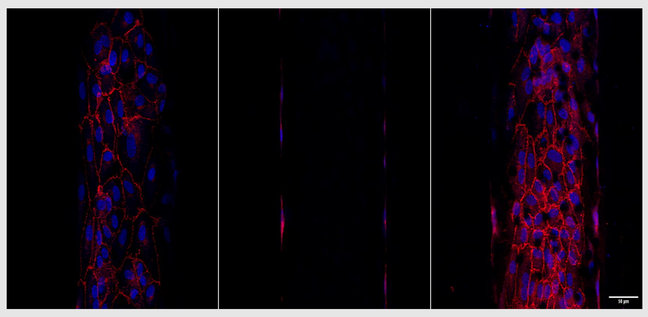


Figure 4. Confocal images inside a 200 µm channel width (Leica SP8, water immersion 25X objective). VE-cadherin (red) and Hoechst (blue) immunofluorescence staining of HUVEC inside the device at day 4 of dynamic culture condition. From left to right: Top, middle and bottom part of the channel

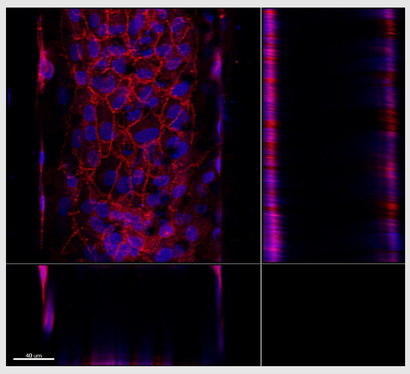


Figure 5. Orthogonal views in a 200 µm channel width obtained by confocal microscopy (Leica SP8, water immersion 25X objective). VE-cadherin (red) and Hoechst (blue) immunofluorescence staining of HUVECs inside the device at day 4 of dynamic culture conditions

- ✓ **Physiological shear stress** between 0,6 and 1,8 dyn/cm², applied step-by-step during 4-5 days
- ✓ The presence of **adherent junctions** troughout the entire microdevice reveal a complete 3D endothelium after 4 days of dynamic culture conditions

Follow in real time the extravasation events

1. Following in real-time tumor cells extravasation

Injection of GFP transfected cancer cells in the upper channel and following of their migration across the endothelium wall

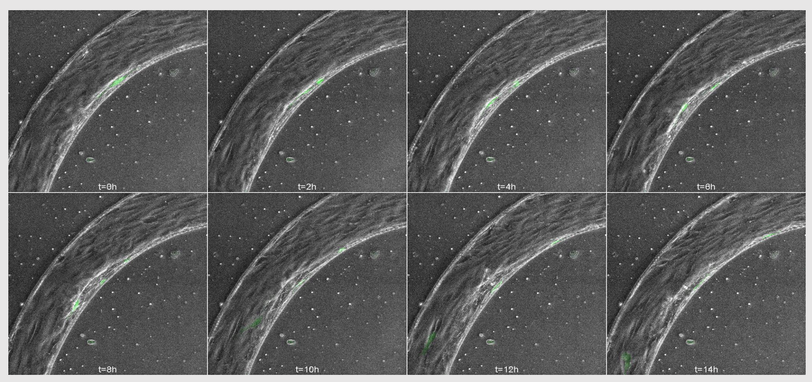


Figure 6. Timeline of interaction between MDA-MB-231-GFP cells and HUVEC endothelium during 14 h. 1h after their injection on HUVEC endothelium, the green fluorescence of GFP MDA-MB-231 cells were recorded

2. Live cells imaging to detect rare extravasation events

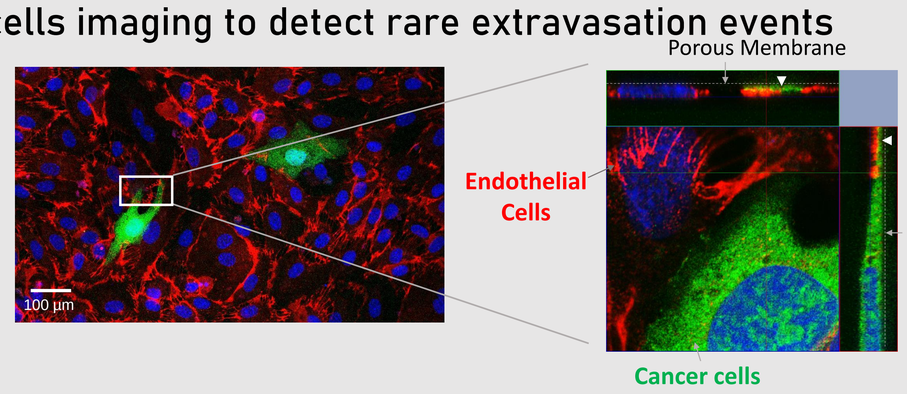
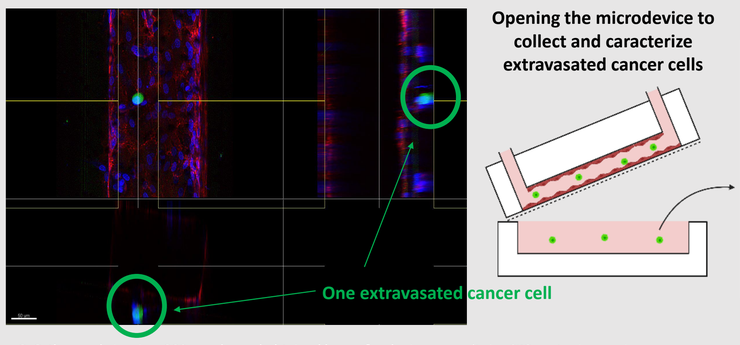


Figure 7. Cancer cells adhesion on endothelial wall. Cancer cells (GFP, green), nucleus (Hoechst, blue), adherent junctions (VE cadherin, red)

3. Collecting extravasated cells



Opening the microdevice to collect and caracterize extravasated cancer cells

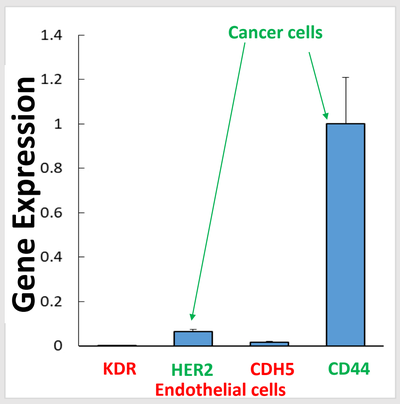


Figure 8. Orthogonal view in a 200 µm channel obtained by confocal microscopy (Leica SP8, water immersion 25X objective). Observation of GFP MDA-MB-231 cells extravasation through a TNF-alpha activated endothelium (Ve-cadherin (red) and Hoechst (blue) immunofluorescence staining. The collecting chamber was filled with 100 ng/ml CXCL12. GFP MDA-MB-231 cells (Green) extravasated through HUVEC endothelium

Figure 9. RT-qPCR expression of KDR (VEGFR2), HER2, CDH5 (VE-cadherin), and CD44 in cells collected in the lower chamber of the device after MDA-MB-231 injection

Outlooks

- Modify the collecting channel to a more complex micro-environment in order to mimic specific targeted organs
- Complete characterization of extravasated cancer cells (stemness, migration, profil…)

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