Cancer Cells-on-Chip 2
State of the Art and Future Developments
28-29 march 2019, Lyon, Rockfeller

Booklet of Abstracts & list of participants

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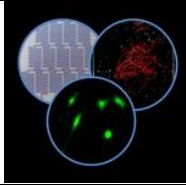
Cancer Cells-on-Chip 2

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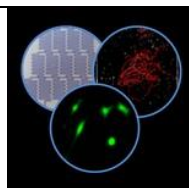
Cancer Cells-on-Chip 2

PROGRAMME

Thursday 28th march 2019, Amphi Hermann:

08H30 Welcome coffee

09H00 - 9H20 Opening speech by Patrick Mehlen (CRCL Director, Responsible Plascan)

09H25 - 11H00 Organoid-on-chip

09H25 - 10H10 Nathalie Picollet D'Hahan (CEA Grenoble)
"Human 3D models in oncology : from organoids to organ-on-chip"

10H15 - 10H35 Frederick de Miollis (JPARC, IEMN, LIMS, Lille)
"Development of a 3D in vitro *microfluidic co-culture system to study tumor-stroma interactions and drug resistance of pancreatic adenocarcinoma"*

10H40 - 11H00 Isabelle Maridonneau-Parini (IPBS, Toulouse)
"Facilitation of tumor cell invasion by macrophage migration"

11H00 - 11H30 Coffee Break + Poster session

11H30 -13H05 Organ-on-chip

11H30 - 12H15 Stéphanie Descroix (Institut Curie, Paris)
"Cancer on chip: from bioanalyis to organ on chip"

12H20 - 12H40 Lilandra Boulais (LBB, Compiègne)
"Cryogel integrated microchip : towards a human 3D model of liver cancer"

12H45 - 13H05 Gaëlle Récher (LP2N, Bordeaux)
"Cell self-organisation in confined environment, reconstructing prototypic & physiomimetic tissues"

13H05 - 14H30 Lunch + Poster session

14H30 - 16H05 New technologies for 3D model

14H30 - 15H15 Charles Baroud (Ladhyx, Paris)
"Understanding three-dimensional spheroid cultures in a high-density microfluidic device"

15H20 - 15H40 Carole Aime (ENS Paris)
"Fibrous culture patches for tumor spheroid formation"

15H45 - 16H05 Jonathan Cottet (Ampère, EPFL, Lyon et Suisse)
"Towards DEP-assisted creation of cell aggregates mimicking tumor"

16H05 - 16H35 Coffee Break + Poster session

16H35 - 17H45 Single Cell approaches

16H35 - 16H55 Anne-Laure Deman (INL, Lyon)
"Array of magnetic micro-traps for CTC sorting in lab on a chip"

17H00 - 17H20 Kyohei Terao (Institut Curie, Paris)
"Cancer cell deformation and recovery within microvascular in vitro *constriction model"*

17H25 - 17H45 Yoran Margaron (Hôpital Saint Louis, CEA Grenoble)
"The specific contributions of ZEB1 expression and RhoA activation to cell polarity remodeling during early stages of TGFβ-induced EMT"

Friday 29th march 2019, Salle des Pas Perdus:

8H30 - 10H25 Biomimetic approaches

08H30-09H15 Jaap Den Toonder (Eindhoven University of Technology, Pays Bas)
"Breast cancer-on-a-chip models for studying cancer metastasis"

09H20-09H40 Anthony Treizebre (IEMN, Lille)
"Microfluidic metastasis-on-a-chip models for investigation of breast cancer stem cells"

09H40-10H25 Stéphane Germain (CIRB, Paris)
3D vascularised tumoroids : towards integration of angiocrine and mechanical signals in vitro

10H25 - 11H00 Coffee Break + Poster session

11H00 - 12H35 Mechanobiology-on-chip

11H00-11H45 Claude Verdier (LIPhy, Grenoble)
"Physical approaches to understand cancer cell transmigration"

11H50-12H10 Patricia Davidson (Institut Curie, Paris)
"Giant nesprins accumulate at the front of nuclei deforming through narrow constrictions"

12H15-12H35 Audrey Prunet (ILM, Lyon)
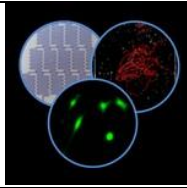*"Soft cell confiner development to decipher the impact of mechanical stimuli on cell"*

12H40 - 13H00 Closing remarks

poster awards

lunch   bags   distribution

Abstracts of oral contributions

Nathalie Picollet D'Hahan

Human 3D models in oncology: from organoids to organ-on-chip

Nathalie Picollet-D'hahan[a], Stéphanie Porte[a], Bastien Laperoussaz[a], Patricia Obeid[a], Frédérique Kermarrec[a], Vincent Haguet[a], Ville Harma[a], Sophie Gerbaud[a], Monika Dolega[a], Donald K Martin[b], Paul Motreuil[a], Hiba Omairi[a], Anthony Berdeu[c], Cedric Allier[c], Xavier Gidrol[a]

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The presentation highlights our major developments in the fields of organoids and organ-on-chip to address issues in fundamental and biomedical research by modeling development and cancer. We illustrate how contemporary miniaturized technologies (e.g. microfluidics, 3D scaffolding, 3D imaging) combined with RNAi-based HTS in 3D organoids would help forming and analyzing reproducible organoids, a prerequisite for applications including disease and cancer modeling and drug development assays. Specifically, we present our approach based on a flow-focusing microfluidic system that encapsulates either single prostatic or mammary cell in Matrigel beads and assay for development of organoids. Furthermore, we developed new imaging technology to monitor live organoids self-assembly and inter organoids cell trafficking. Finally, we illustrate the potential of engineered organ-on-chip devices for creating novel human organ and disease models, with a particular focus on "prostate-on-chip" developments.

Keywords— 3D organoids, μencapsulation, 3D lensfree imaging, 3D scaffolding, prostate cancer, exocrine gland-on chip.

- 3D polyelectrolyte scaffolds to mimic exocrine glands: a step towards a prostate-on-chip platform. Nathalie Picollet-D'hahan *et al. The EuroBiotech Journal*, 180-195, 4 (2), (**2018**).
- Direct transfection of clonal organoids in Matrigel microbeads: a promising approach towards organoid-based genetic screens. Laperrousaz Bastien *et al. Nucleic Acid Res., 1-13* (**2018**).
- Lens-free microscopy for 3D + time acquisitions of 3D cell culture. Anthony Berdeu *et al. Scientific Rep.*8, 16135 (**2018**).
- Deciphering cell intrinsic properties: A key issue for robust organoids production. Nathalie Picollet-D'hahan *et al. Trends in Biotech 35(11):1035-1048* (**2017**).
- A 3D toolbox to enhance the physiological relevance of human tissue models. Nathalie Picollet-D'hahan *et al. Trends in Biotech*. 34 (9), 757-769 (**2016**).
- Controlled 3D culture in Matrigel microbeads to analyze clonal acinar Development. Dolega ME *et al. Biomaterials* 52, 347-357 (**2015**)

Frederick de Miollis

Development of a 3D in vitro microfluidic co-culture system to study tumor-stroma interactions and drug resistance of pancreatic adenocarcinoma

Frédérick de Miollis[1,2,3], **Romain Vasseur**[1], **Isabelle Van Seuningen**[1*] **& Vincent Senez**[2,3*]

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Introduction: Pancreatic cancer (PC) is one of the most deadly cancers in western countries with a very bad prognosis due to a strong resistance to chemotherapeutic treatments and a lack of efficient therapeutic tools. The physical microenvironment of pancreatic tumors is complex and has an important impact on the biology of cancer cells and their response to chemotherapeutic agents. In our work, we have studied the conditions to allow long term and stable co-culture of PC cells with fibroblasts that is a preliminary step before analyzing their interactions and possible effects on drug resistance.

Methods: Our chip is based on a central microchannel confined by micropillars and two lateral microchannels with an internal height of 100 µm. It is fabricated using micromachining techniques, by obtaining the Polydimethylsiloxane (PDMS) replica from a Si/SU-8 master with CHF_3 deposit. It is then bonded on a microscope glass slide to allow high spatial resolution microscopy. The central channel is filled with PC cells (MiaPaCa-2, Panc-1, Capan-2) and/or Fibroblasts (CAFs or PSC) mixed in hydrogel (Matrigel) and the lateral channels allow controlled alimentation with cell culture medium containing either drugs or oncogenic molecules. Cell co-culture has been validated by studying cell morphology and phenotypic characteristics (marker expression by Western-blotting).

Results: We have developed a 3D co-culture chip that mimics the tumor microenvironment and integrates PC cells with PSC in proximity within a hydrogel scaffold. Morphology (epithelial and mesenchymal) has been confirmed by light microscopy observation. Expression of epithelial (MUC1 mucin, CK8/CK18 cytokeratins, E-cadherin) and mesenchymal (vimentin, α-SMA, fibronectin) markers has been confirmed by western-blotting and Immunofluorescence. We also tested one important culture parameter for our device: the velocity of the culture medium to control and optimize the gradient of nutrients and drugs.

Conclusion: We have validated our co-culture system both at morphological and phenotypic levels. We will now continue with studying proliferation/migration properties and drug response.

Isabelle Maridonneau-Parini

Macrophages are towing tumor cells to escape spheroids

Maridonneau-Parini Isabell[1], Guiet Romain, Cougoule Céline, Le Cabec Veronique

Institut de Pharmacologie et Biologie Structurale, CNRS UMR5089, Toulouse (France)

Tumor-associated macrophages are known to amplify the malignant potential of tumors by secreting a variety of cytokines and proteases involved in tumor cell invasion and metastasis. Recently, we have shown *in vivo* that macrophages use the protease-dependent mesenchymal migration mode to infiltrate tumors and the amoeboid mode in the tissue surrounding the tumor (Gui, P. et al. *Cancer Immunol. Res.* 2018). Here we examined whether the macrophage migration process facilitates tumor cell invasion. To address this question, we used cell spheroids of breast carcinoma SUM159PT cells infiltrated or not by macrophages. Individually, SUM159PT cells were able to invade Matrigel in a matrix metalloprotease (MMP)-dependent manner but, when organized as spheroids, they lost this capacity and did not spontaneously invade Matrigel surrounding spheroids . Tumor cell invasiveness was restored when spheroids were infiltrated or in contact with macrophages that remodelled the matrix with proteases. We observed that macrophages formed paths (tunnels) in which SUM159PT cells used the protease-independent amoeboid migration mode to follow macrophages in close proximity. The number of tumor cells invading Matrigel was reduced when mesenchymal migrationdefective macrophages were used. Thus, in addition to the well-described paracrine loop between macrophages and tumor cells, macrophages can also contribute to the invasiveness of tumor cells by remodeling the extracellular matrix, opening tunnels that are used by tumor cells to colonize the surrounding tissues in an MMPdispensable manner.

Stéphanie Descroix

Cancer on chip: from bioanalyis to organ on chip

Physico-Chimie Curie, Institut Pierre-Gilles de Gennes pour la Microfluidique, Paris

Microfluidics is considered by researchers and clinicians as a disruptive technology in cancer research. The potential of microfluidics has already been well established regarding the analysis of circulating biomarkers. More recently its ability to unravel complex mechanisms of cancer cells invasion, proliferation and interaction with their microenvironment has been demonstrated. Here I will present how the combination of magnetic particles and microfluidics is valuable for circulating biomarkers analysis. Then, in a second part, I will introduce new technologies developed to reproduce tumor microenvironment on chip.

Lilandra Boulais

Cryogel integrated microchip:

towards a human 3D model of liver cancer

Lilandra Boulais[1], Rachid Jellali[1], Ulysse Pereira[1], Patrick Paullier[1], Eric Leclerc[1],Sidi A.Bencherif[1,2,3], Cécile Legallais[1]

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Recently, a number of studies have highlighted that the cell microenvironment plays a critical role in various biological processes. In this context, 3D cell culture systems have been the focus of much attention to mimic more closely the natural environment found in vivo. To this end, we have developed an innovative technology combining biomaterials and microfluidics. A polydimethylsiloxane (PDMS) microchip containing a macroporous alginate hydrogel was designed for hepatocyte culture to create a liver tissue.

The microfluidic device was successfully engineered to ensure a homogeneous perfusion while providing a micro-sized cell culture chamber in 3D. Inside this microchip device, alginate was covalently crosslinked at subzero temperatures to create a macroporous scaffold which was then coated with collagen before cell seeding. HepG2C3a were cultured in this device in dynamic conditions (10µL/min) for 7 days.

The physical properties of the integrated cryogels were characterized: the SEM observations showed a macroporous structure with an average pore size of 100µm (**Fig.1**). The cryogel presented a high degree of pore connectivity and swelling ratio allowing cell culture medium circulation through its 3D structure.

The HepG2C3a cells adhered on alginate cryogel and spread inside this macroporous scaffold. The SEM observations showed that after 7 days of culture, these proliferative cells formed 3D structures which remained inside the microchip after the removal of the alginate cryogel by alginate lyase (**Fig.2**).

Looking into the future, this innovative microchip may be a promising device to improve predictive toxicology and to develop personalized medicine.
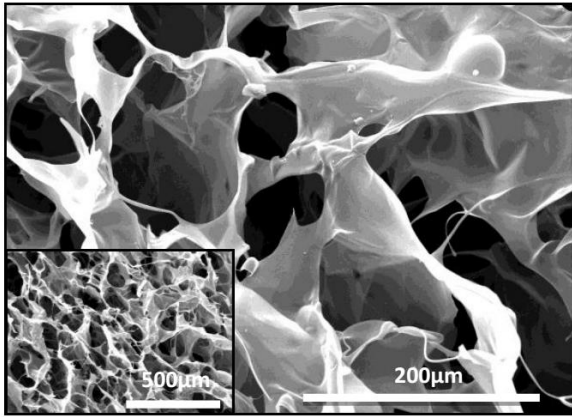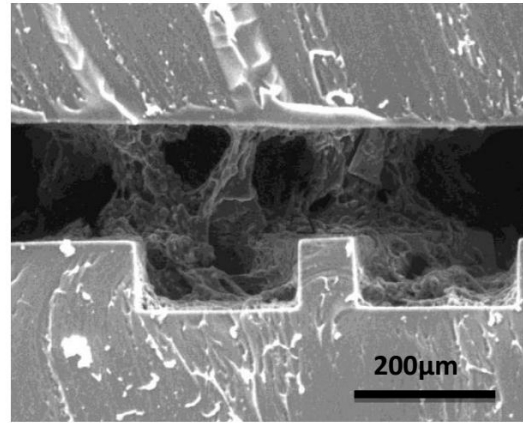
Fig.1 SEM image of alginate cryogel

Fig.2 SEM image of HepG2C3a at day 7 in dynamic conditions (10µL/min) after removal of alginate cryogel

Gaëlle Récher

Cell self-organisation in confined environment, reconstructing prototypic & physiomimetic tissues

Recher, Gaëlle[1,2,3], Naveen Vijayan Mehkileri[1,2,3], Mombereau, Amaël [1,2,3], Hélaine, Nelson [1,2,3], Nassoy, Pierre [1,2,3]

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https://biof-lab.org/

Cells and tissue morphogenesis occur in tight interrelation with a complex environment which embrace a multiplicity of biological/physical/chemical parameters. Physiologically relevant *in vitro* models that tend to take that complexity in consideration are difficult to tackle.

I will show how combining tailored approaches [micro-fluidics + cell engineering + microscopies + image analysis] in different cells and tissues context opens avenues for generating functional and physiologically relevant models for both the establishment of cancer models & the up-scaling of tissue engineering.

The Cellular Capsule Technology serves for the generation of spherical B-cell Lymphoma niche organoids (combining stromal cells & tumour cells) and tubular blood-vessel-like organoids (that we named vesseloids) that are independently generated and characterised (including with live cell imaging). Future directions will then consist in combining both approaches to generate vascularised organoids.

Charles Baroud (Ladhyx, Ecole Polytechnique)

Interactions between 3D spheroids and immune cells on a microfluidic device

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In this presentation I will describe our microfluidic platform for the formation, manipulation, and observation of 3D spheroids. The system allows us to obtain vast quantities of image data, resolved on the single-cell level, that provide a mapping of the biological state of the cells within the 3D structure. The data thus generated can be resolved on the scale of the population, of each spheroid, or on the scale of individual cells in the spheroids, leading to what we term "multiscale cytometry" [1].
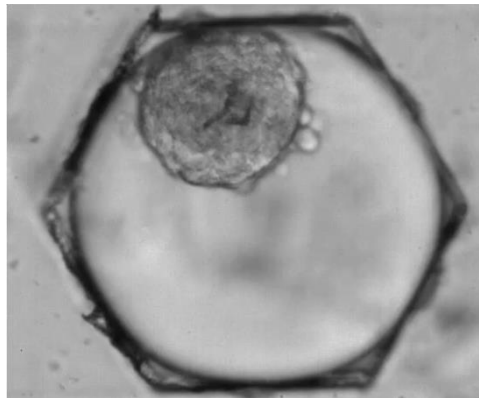

Figure 1: Image of a spheroid within a microfluidic droplet. A single chip contains 250-500 such spheroids.

Further microfluidic developments also allow us to submit the spheroids to different conditions in each droplet, using a fusion step with a second library of drops. In this way we can test the response of spheroids to different drug concentrations. The time-resolved measurements evidence the presence of a collective response, on the scale of the whole spheroid, to the drug. These show for instance an avalanche of cell deaths, once enough individual cells have died following the exposure to the drug [2].

Finally I will end by showing some recent work that we are performing to develop an immuno-therapy model on a chip. Time-lapse microscopy here allows us to observe individual T-cells interacting with a cancer spheroid.

References:

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Carole Aime

Fibrous culture patches for tumor spheroid formation

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Cell-based assays that recapitulate the tumor specificity are necessary to understand tumor growth and evaluate the efficiency of anti-cancer drugs [1]. We have developed culture patches using electrospinning for maintenance and differentiation of human induced pluripotent stem cells (hiPSCs). [2-4]. Here we adapt those patches for large size tumor spheroid formation that is useful for anticancer drug studies. Electrospinning is particularly interesting for cell biologists because it provides a more natural 3D microenvironment to cells than is possible with other techniques, with the formation of a net-like structure made of cross-linked fibers mimicking the extracellular matrix. A honeycomb poly(ethylene glycol) diacrylate (PEGDA) microframe defined by photolithography is used. After gold-coating of the frame, different (bio)polymers are electrospun to produce layers of nano to microfibers. Processing parameters are constantly adjusted to assess the formation performance of spheroids on fibrous substrates. Systematic studies are expected to provide a better and more complete description and mechanistic understanding of spheroid formation and anti-cancer drug effect on tumors.

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Jonathan Cottet

Towards DEP-assisted creation of cell aggregates mimicking tumor

Jonathan Cottet [a,b]**, Olivier Fabregue**[a]**, Julien Marchalot**[a]**, Riccardo Scorretti**[a]**, Laure Franqueville**[a]**,
François Buret**[a]**, Marie Frénéa-Robin**[a] **and Philippe Renaud**[b]

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Cell aggregates are useful intermediary models between the single cell and the tissue to understand how electric properties are modified in a multicellular construct. Such information can help to improve electrochemotherapy, the use of electric fields to enhance the local delivery of chemotherapeutic agents directly into cancer cells. While in vitro studies performed on isolated cells provide useful information on how field pulses induce biological membrane permeabilization, more realistic 3D in vitro models are required to mimic the behavior of groups of cells in a tumor or a tissue and optimize electropulsation protocols.

We present a microfluidic platform allowing dielectrophoresis-assisted formation of cell aggregates of controlled size and composition under flow conditions. When specific experimental conditions are met, negative dielectrophoresis (nDEP) allows efficient concentration of cells towards electric field minima and subsequent aggregation. This bottom-up assembly strategy offers several advantages with respect to the targeted application: first, DEP offers precise control of spatial cell organization, which can be adjusted by optimizing electrode design. Then, it could contribute to accelerate the establishment of cell-cell interactions by favoring close contact between neighboring cells. The trapping geometry of our chip is composed of 8 electrodes arranged in a circle. The microfluidic chips were obtained using a novel method of fabrication involving the alignment of a microchannel layer made of PDMS on top of Ti/Pt electrodes[1]. Several parameters have been tested in simulations to find the best configurations for trapping in flow. Those configurations have been tested experimentally with both polystyrene beads and HEK cells as illustrated in Figure 1.
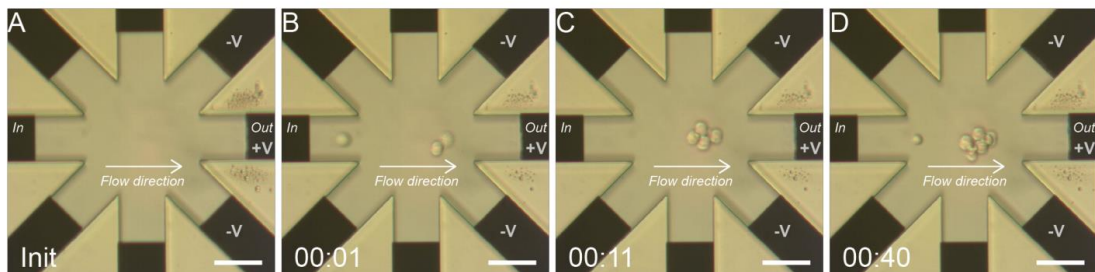

Figure 1 HEK aggregation in flow conditions using 3 electrodes. Time stamps in mm:ss format. Scalebar 50 µm

The final design and experimental setup have been optimized to trap cells and release the created aggregates on demand. To be able to prevent new cells from entering the trapping chamber, the experimental setup was fully controlled with LabVIEW (National Instruments)[2]. The aggregate proved to be stable after 5 minutes of cell-cell contact.

The here presented set-up offers the possibility to further investigate interactions between different cell types by creating composite aggregates with a known composition. Aggregates created on-chip could also be part of a Lab-on-a-chip system by adding the possibility to electroporate the created aggregates and observe their properties before and after electroporation. Simultaneously the collective creation of larger cell aggregates of regular size and shape is also investigated[3].

References

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Anne-Laure Deman

Array of magnetic micro-traps for CTC sorting in lab on a chip

Lucie Descamps[1], Samir Mekkaoui[1], Emmanuelle Laurenceau[1], Marie-Charlotte Audry[1], Jessica Garcia[2], Lea Payen[2], Damien Le Roy[3] and Anne-Laure Deman[1]

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In these last decades, the count of circulating tumor cells (CTCs) from total blood has been clearly associated with bad prognosis in many cancer types. In particular, Epithelial-Mesenchymal Transition (EMT) phenotype of CTCs was associated with an increase of the capacity of invasiveness, immune escape and metastasis [1]. In addition, CTC-clusters demonstrated increased metastatic potential compared to single CTCs and their presence is strongly correlated with a dramatically shorter overall survival time. Thus, CTCs' monitoring can serve to analyze treatment response and carry out personalized therapy [2,3]. However, recovering CTCs from blood samples is a great challenge regarding their low abundance: 1 to 3000 CTCs per mL, among the large amount of blood cells, i.e. 10^9 red blood cells (RBCs), 10^7 white blood cells (WBCs) per mL.

Microchip devices coupled to microfluidic approach are a proven technology for cellular handling as they can offer precise spatial and temporal control in a miniaturized environment compatible with cell or cell cluster size. Various functions (detection, focusing, mixing, counting, lysing) can be implemented on a single chip contributing to the analysis of individual cells on an integrated platform for complete lab on a chip application. Moreover, these devices can be easily made in polymers such as polydimethylsiloxane (PDMS) using microfabrication tools, which results in lower cost. Among the different approaches to isolate CTC, immuno-magnetic based micro-devices, combine benefit from microfluidic format for rare cell handling, highly selective and versatile sorting method, high purity devices and recovery of living cells for subsequent analysis (drug testing, cell culture…)[4,5]. The implementation of magnetic functions is based on magnetophoresis, and higher forces can be reached when micromagnets are integrated in microchannels. However, it requires complex and costly fabrication process, and face challenges related to the heterogeneous integration of metals with polymers, mainly polydimethylsiloxane (PDMS), such as tedious alignment procedures for locating the traps in the channels, and tightness issues.

To overcome these drawbacks, we propose a novel technology to obtain arrays of efficient magnetic micro-traps. The traps consist in high aspect ratio agglomerates of magnetic particles diluted in PDMS matrix. This novel technology, inspired by composite-polymer, is low cost and requires simple fabrication process that breaks with standard microfabrication approaches [6]. Micro-traps are obtained by applying a magnetic field during the composite reticulation [7]. We developed magnetic micro-traps, based on NdFeB particles, and obtained arrays of 600 to 1000 magnetic micro-traps/mm^2, depending on the microparticle's concentration, 4 to 11 µm in diameter. Figure 1 a) and b) show reconstructed 3D views from X-ray tomography characterization of magnetic micro-traps, and a schematic of the microfluidic device. Optimal conditions to elaborate efficient micro-traps were evaluated using model magnetic micro-beads (12 µm in diameter). Then we performed preliminary experiments to specifically immuno-capture WBCs on these micro-traps. Magnetic nanoparticles (300 nm in diameter) were functionalized with DL650 labeled anti-CD45 antibody. Immuno-magnetic nanoparticles were incubated with WBCs, at a concentration of 10^3 nanoparticles per WBC, for 1 hour and injected at 1mL/h in the microfluidic device containing the array of magnetic micro-traps (500 GB/µL) (Figure 1 (c)). Magnetic micro-traps were able to capture magnetically immuno-labeled WBC

as reported on Figure 1(d). These results are very promising for the immuno-magnetic sorting of CTCs using these microtraps either by positive selection through the magnetic immuno-capture of CTCs, or by negative selection through the magnetic immuno-capture of leukocytes in the blood sample.
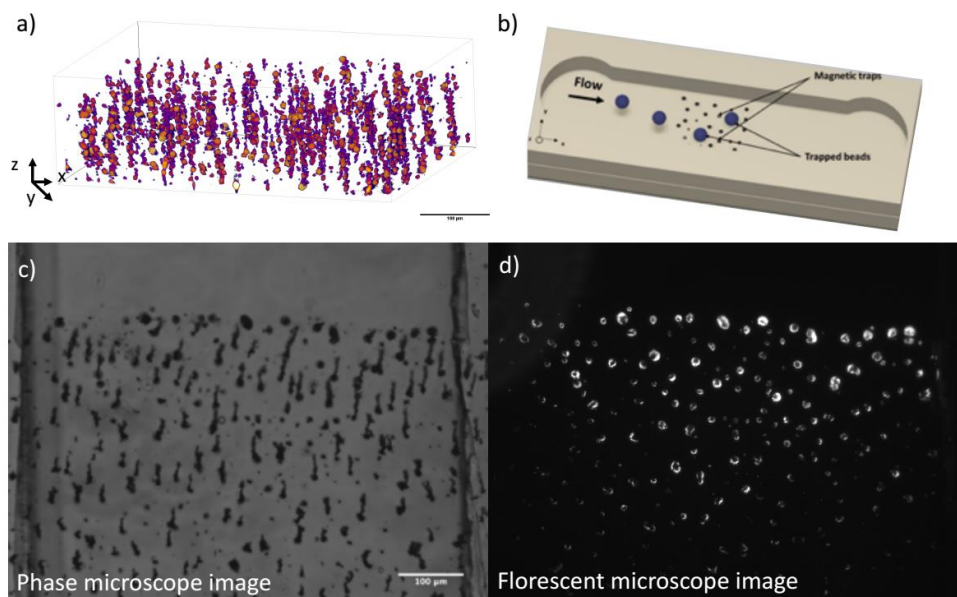

Figure 1 : (a) Reconstructed views, 3D and XY plane, from X-ray tomography performed on a volume of 540 x 330 x 72 μm³ of one composite membrane (Iron-PDMS, 5 wt%), (b) Schematic of the device (for hard micro-traps the magnet underneath the device is removed), (c,d) phase and fluorescent microscope images of immune-magnetically labelled WBCs (DL650 labeled anti-CD45 grafted on 300 nm magnetic nanoparticles) captured on hard magnetic micro-traps .

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Kyohei Terao

Cancer cell deformation and recovery within microvascular in vitro constriction model

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To investigate the impact of a microvascular constriction on circulating tumor cells (CTCs), we developed a microfluidic model that allows *in situ* visualization of cell deformation and recovery. The dynamics of cancer cells was successfully observed during and after passing through a constriction, enabling us to evaluate the recovery time of cell and nucleus shapes.

The processes leading to metastases involve survival of CTCs in blood circulation, arrest, and extravasation. Although these have been studied intensely, the impact of harsh circulation of microvascular network on these events is little understood, because of technical limitations of *in vivo* analysis and a lack of *in vitro* model reproducing the microenvironment. Thus, we developed a microfluidic device to induce cell deformation by micro-constriction mimicking *in vivo* situation and to allow observation of recovery from the deformation.

A cancer cell passing through an *in vitro* constriction is deformed to have an elongated shape (Fig. 1). The cell after passing the constriction is trapped by a flow at a microstructure which we named "single cell nest", and observed under an optical microscope. Single cell nest adjacent to a constriction enables us to visualize cell deformation and recovery simultaneously in a field of microscope view. To increase the throughput, we arranged an array of single cell nest (Fig. 2). A nest is designed to accept single cell, thus cells passing through constrictions bypath the nests occupied with the other cells. Simulation result shows the flow paths in a constriction and gaps between microposts, which introduce cells to the constriction and to the trap sites of single cell nests. Pressure applied between the inlet and the outlet of the channel drops mostly at the constrictions, indicating that the pressure pushes cells in the constrictions effectively to deform them, whereas it allows recovery for the cells resting in single cell nest array, which feel the pressure less than 1% of the pressure applied in constrictions. This configuration deforms cells effectively in constrictions and reduces physical damage to the cells in single cell nest array.

Cancer cell line MDA-MB-231 ($\phi \sim 16$ μm) was used in our experiments. The device was fabricated with PDMS to have the narrowest gaps and the channel height of 6 and 15 μm respectively, confining deformation along z-axis in the constrictions. Cells in the constrictions were elongated to pass the narrow gaps (Fig. 4). After passing there, cells were trapped and recovered gradually to have spherical shape. Deformation of a cell was quantified with the aspect ratio of an ellipsoid fitted to the cell shape. The time course shows a decay of deformation, which is fitted well with an exponential decay curve (Fig. 5). We defined the time constant of the curve as recovery time, and estimated it to be 9.4 ± 4.4 s (mean ± s.d.). The recovery is substantially slow compared to that from small deformation induced by AFM tip, optical traps, and magnetic beads, whereas comparable to the values obtained by micropipette experiments for large deformation. Note that the large cell recovery time observed here is several orders of magnitude higher than the transit time of cells between two successive microvascular constrictions. Therefore, cell shape memory phenomenon should be taken into account in the understanding of CTCs behavior within the blood circulation.

Fluorescence staining successfully visualized the deformation and recovery of nucleus shape (Fig. 6). Nucleus recovery took place much faster than cell (Fig. 7). *In situ* visualization of intracellular dynamics using fluorescently-labeled molecules will lead to further understandings of the damage and recovery of biological processes that CTCs experience in microvascular constrictions.
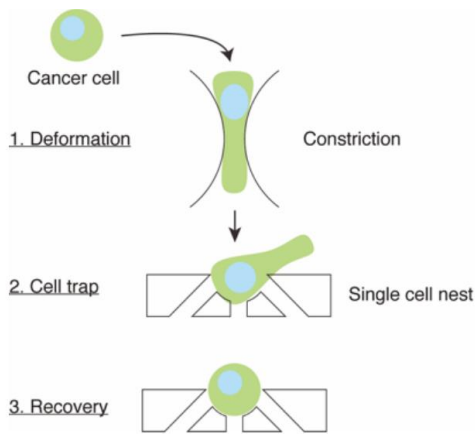
Fig. 1 Concept of vizualizing cancer cell deformation and recovery within in vitro constriction model.

Fig. 2 Microfluidic device with 2-types of constrictions and single cell nest array.

Fig. 3 Simulation results of the flow in the microfluidic device.

Fig. 4 Time-series of cell deformation and recovery.

Fig. 5 Timecourse of shape recovery. Inset shows a histogram of the recovery time.

Fig. 6 Time-series of nucleus deformation and recovery.

Fig. 7 Change of recovery state in accordance with time.

Yoran Margaron

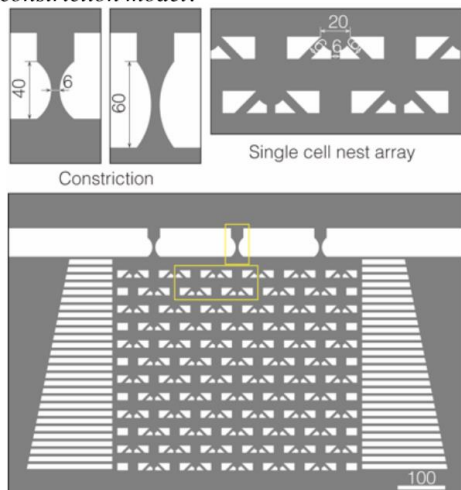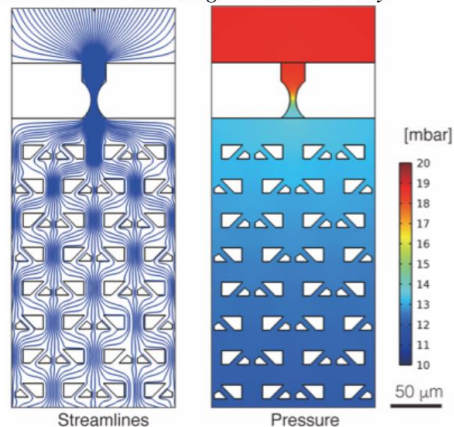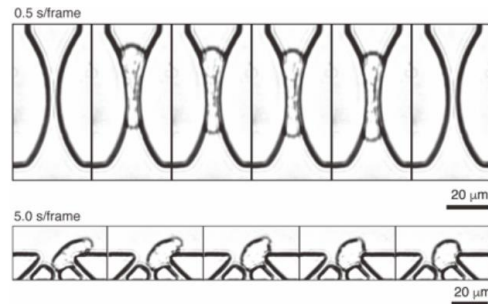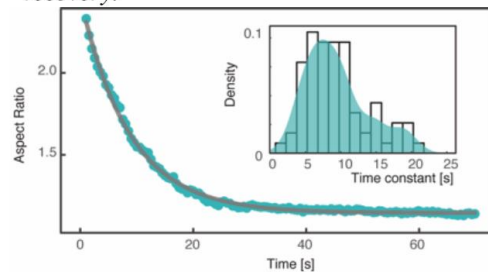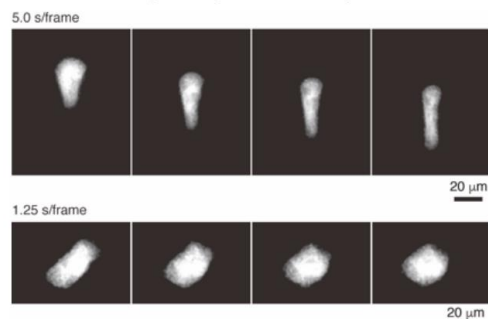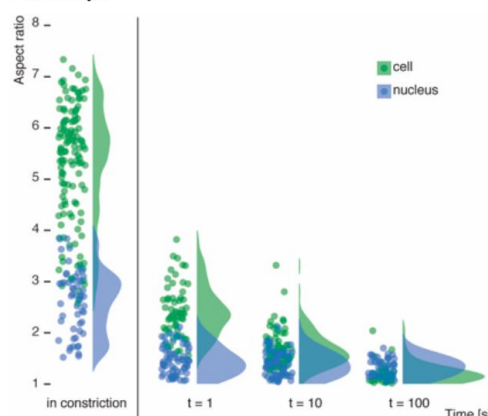## *The specific contributions of ZEB1 expression and RhoA activation to cell polarity remodeling during early stages of TGFβ-induced EMT*

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[2] Université Grenoble-Alpes, CEA/INRA/CNRS, Biosciences & Biotechnology Institute of Grenoble, LPCV, CytoMorpho Lab, 17 rue des Martyrs, 38054 Grenoble, France.
[3] Université de Lyon, Université Claude Bernard Lyon 1, INSERM 1052, CNRS 5286, Centre Léon Bérard, Cancer Research Center of Lyon, Lyon, France.

Epithelial-to-mesenchymal transition (EMT) is a dynamic and reversible process ultimately driving cell plasticity and the dissemination of tumor metastatic cells. Upstream regulation of EMT encompasses pleiotropic signaling events leading to profound remodeling of cell cytoskeleton. TGFb is a potent inducer of EMT with a broad spectrum of down-stream effectors. It has notably been shown recently to trigger the reversal of the orientation of cell polarity, which primes cells for scattering. However the specific contribution of TGFb downstream effectors to this polarity reorientation is not known. Here we used a minimal epithelium model to perform a quantitative analysis of the regulation of cell architecture and polarity during the early stages of EMT in response to Zeb1 expression. We found that contrary to TGFb, which induces the complete reversal of the orientation of cell polarity, the expression ZEB1 only disrupts the original epithelial orientation without inducing a defined front-rear mesenchymal polarization. Both Zeb1 expression and stimulation of TGFb destabilized microtubules and triggered the repositioning of the centrosome-microtubule network from the inter-cellular junction to the geometric cell center. By contrast, Zeb1 and TGFb had opposite impact on cell contractility and the reorganization of the actin network. TGFb stimulated the production of traction forces and promoted the rearward displacement of the nucleus toward inter-cellular junctions, whereas Zeb1 relaxed the actin network and kept the nucleus close to cell geometrical center. Down-regulation of the ROCK-dependent cell contractility in cells treated with TGFb or its up-regulation in cells over-expressing Zeb1 reversed both effects. As a consequence, it appeared that TGFb activation and Zeb1 expression triggered cell scattering but TGFb reduced individual cell migration speed whereas Zeb1 increased it. Altogether these results show that TGFb-induced EMT results from the loss of baso-apical epithelial polarity in response to Zeb1 expression and the acquisition of a front-rear mesenchymal polarity downstream of ROCK activation.

Jaap Den Toonder

Breast cancer-on-a-chip models for studying cancer metastasis

Eindhoven University of Technology

Microfluidics technology offers the possibility to create devices in which chemical, mechanical, and physical conditions can be precisely controlled. This makes it possible to realize well-defined micro-environments to realize advanced multi-cellular culture systems to investigate tissue and organ function, and to recreate aspects of diseases to understand processes and mechanisms in disease progression. Microfluidic chips, therefore, are very suitable for studying the effect of microenvironment on cancer development, and particularly on metastasis, the spreading of cancer cells throughout the body.

Most cancer deaths are caused by secondary tumors formed through metastasis, yet due to our limited understanding of this process, prevention remains a major challenge. One of the main difficulties in elucidating the mechanisms that underlie metastasis is the role of cues from the tumor microenvironment (TME) in directing cancer cell behavior [1]. These cancer-cell extrinsic cues are typically not present in conventional in vitro models, or not controlled in in vivo models. Therefore, we turn to microfluidic Cancer-on-a-Chip (CoC) models in order to study the effect of TME cues on cancer cell behavior.

We have developed a number of CoC models, to study the effects of different microenvironmental cues on cancer cell invasion and migration, two important steps in metastasis. Fig. 1(a) shows a multilayer microfluidic chip with an integrated extracellular matrix (ECM) layer, that we have used to study cancer cell invasion and migration in ECM with controlled properties, and in the presence of a chemotactic gradient [2]. Using a microfluidics-based 3D culture model mimicking breast cancer, we can follow the process of cancer invasion live, see Fig. 1(b). Fig. 1(c) depicts a microfluidic chip with fully controlled oxygen gradient to study the effect of this gradient on cancer cell migration [3].

In this lecture, I will explain details of the design and application of these models and present the main results obtained with them, and I will give an outlook on future developments of CoC.
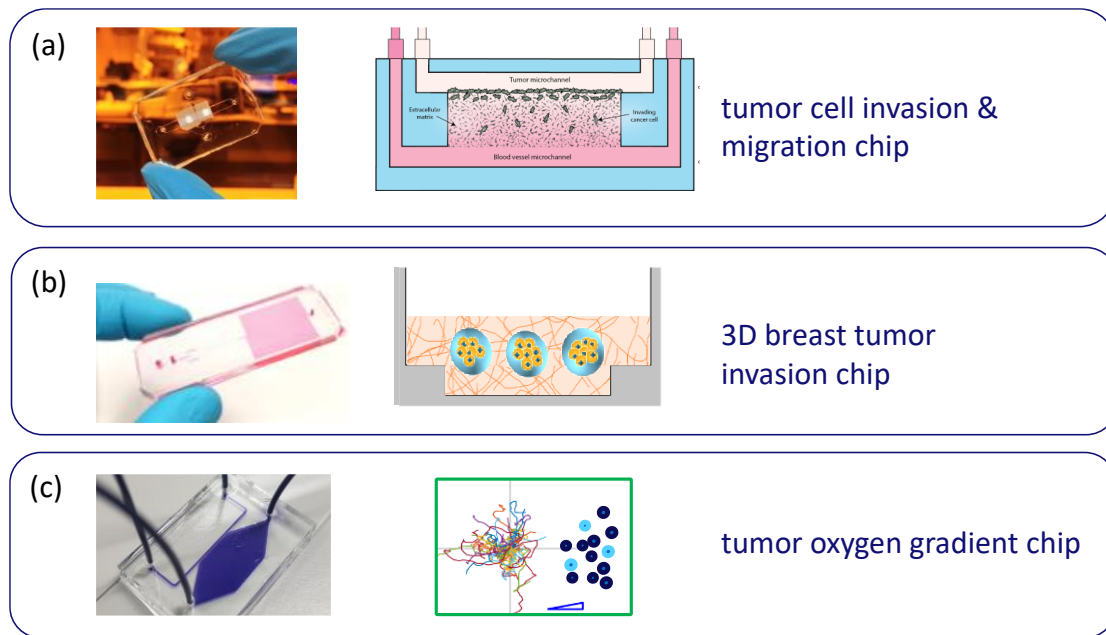
Figure 1: Our microfluidic Cancer-on-Chip models. (a) A multilayer microfluidic chip with an integrated ECM layer, that can be used to study cancer cell invasion and migration in ECM with controlled properties, and in the presence of a chemotactic gradient. (b) A microfluidics-based 3D culture model mimicking breast cancer in which cancer invasion can be followed live. (c) A microfluidic chip with fully controlled oxygen gradient to study the effect of this gradient on cancer cell migration.

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Anthony Treizebre

Microfluidic metastasis-on-a-chip models for investigation of breast cancer stem cells (BCSCs)

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ABSTRACT

This paper presents an original biomimetic microfluidic device like a powerful platform for the identification and understanding of the metastatic algorithm. We propose to study the metastatic potential of breast cancer cell focusing on cancer stem cells (CSCs) and non-CSCs, using live imaging, within a device allowing the understanding of flow effect by a complex bifurcation network and the modulation of the metastatic microenvironment.

KEYWORDS: Metastasis-on-Chip, Breast Cancer Stem Cells, Biomimetic Devices

INTRODUCTION

Most of work done in this area focus on cancer cell adhesion and extravasation or their interaction with the environment [1]. However, few studies combine both and focus on cancer cell heterogeneity, as CSC vs. non-CSC [2]. Indeed, recent clinical data support the view that many solid cancers are organized hierarchically with a small number of cancer stem cells (CSCs) able to recreate a tumor [3]. Clinically, breast CSCs (BCSCs) have been associated with higher rates of recurrence and metastasis [4]. As CSCs is the only cells able to regenerate a tumor, their implications in metastatic development have been suggested. Recent studies have shown that CSCs could rise from non-CSCs under specific conditions as hypoxia or radiation treatment. We propose to study the metastatic potential of breast cancer cells focusing on CSCs/non-CSCs using live imaging, within a device allowing the understanding of flow effect by a complex bifurcation network and the modulation of the metastatic microenvironment. We want to assess if metastasis arise from CSCs able to extravasate and resume growth and/or if metastasis arise from non-CSCs that will reprogram into CSCs once extravasated in appropriate environment [5].

EXPERIMENTAL

This PDMS device is composed of two chambers linked by a porous membrane. The first is dedicated to reproduce biomimetic blood vessels [6], the second to generate a specific tunable metastatic niche (Fig.1). In the top layer, the entrance channel is 800µm wide and divided into two half-width channels, up to 8 channels of 100µm in the center with a height of 50µm. The bottom layer is composed of a single channel, 500µm wide and 150µm thick, weaving through the chip and placed below each straight top channel. Upper and lower chamber are separated by a polycarbonate porous membrane with pores sizes of 8µm [7] (Fig.2).
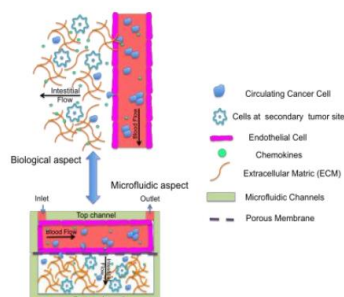

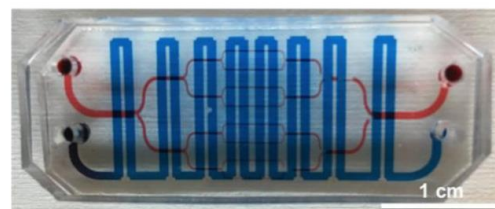*Figure 1: Microfluidic metastasis-on-a-chip models for investigation of cancer extravasation*

Figure 2. Biomimetic microfluidic device illustrating the microvasculature network (red) and the second network used to apply metastatic microenvironment (blue).

This device is placed under microscope including an environmental chamber for time-lapse imaging capability (Fig.3). To generate 3D functional biomimetic blood vessels, the inner surfaces are coated with collagen (100µg/ml) and fibronectin (100µg/ml). Then, Human Umbilical Vein Endothelial Cells (HUVECs) are injected at high density (8×10^5cells/15µl) inside the top chamber.
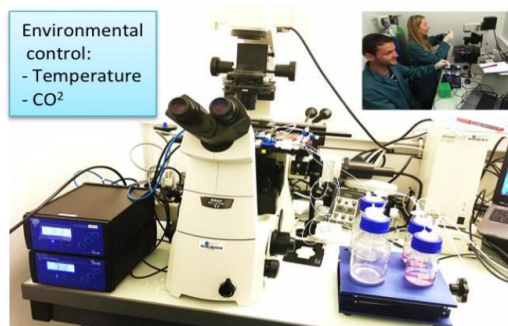

Figure 3. Experimental setup under microscope with environmental chamber for time lapses observation.

RESULTS AND DISCUSSION

After 4h, culture medium (Medium 200, Gibco) is perfused for up to 3 days. Anti-CD31 immunofluorescence staining combined with confocal microscopy observations reveals a tight and confluent endothelial monolayer covering all channel inner surface (Fig.4). We have also evaluated cancer cell adhesion on HUVECs monolayer. This monolayer was activated with 10 ng/ml TNF-α for 24h. Metastatic human breast adenocarcinoma cells (MDA-MB-231) are injected inside the top channel under flow conditions. Our results showed that a small proportion of these cancer cells remained attached to the activated endothelium and are able to grow (Fig.5).
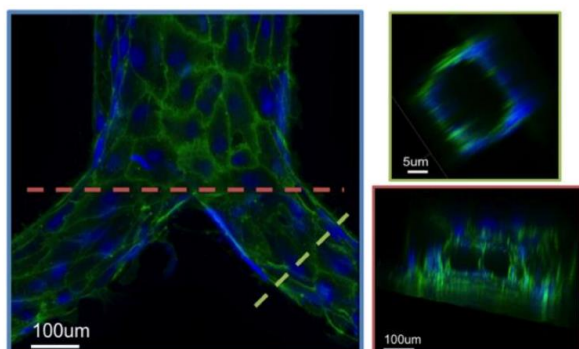
Figure 4. Confocal microscopy images of the microfluidic channel revealed a 3D endothelial monolayer. Immunofluorescence staining: Hoechst (blue) and anti-CD31 (green).
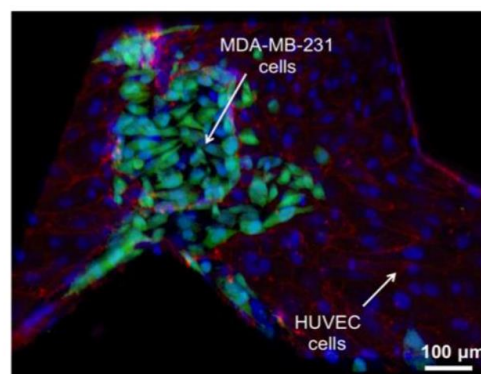
Figure 5. Confocal microscopy images of MDA cells arrested and proliferating on TNF-α activated HUVECs monolayer Immunofluorescence staining: Hoechst (blue), anti-CD31 (red) MDA-MB-231 cells (green).

As our device is functional for metastasis analysis, we will inject cells containing reporter to identify CSCs from non-CSCs (mNeptune under the control of ALDH1A1 promoter). Phenotypes of adhering, extravasating and/or proliferating cells will be characterized through time according to the microenvironment embedded in the bottom chamber.

CONCLUSION

We have designed an innovative microfluidic device allowing to study the metastatic potential of breast cancer cells focusing on CSC/non-CSC under flow conditions using live cell imaging. This microfluidic chip enables the modulation of the metastatic microenvironment through the bottom chamber in order to study its

impact on cancer cells adhesion, extravasation and proliferation. The next step will be to understand the link between cancer cells extravasation and invasion capabilities, and CSCs/non-CSC phenotypes.

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Stéphane Germain

3D vascularised tumoroids: towards integration of angiocrine and mechanical signals in vitro

CIRB College de France - UMRS INSERM U1050 - CNRS 7241 - Paris

Accurate mimicry of human tumorigenesis is extremely difficult thus questioning the usefulness of existing *in vitro* and *in vivo* models for therapeutic translation in humans. In particular, Hepatocellular carcinoma (HCC) represents the 3rd cause of death by cancer, arising in up to 80% of patients with chronic liver diseases and cirrhosis, but there are no models able to recapitulate diversity, assess prognosis, test drugs and predict efficacy. According to the current therapeutic guidelines, transarterial chemoembolization (TACE) or systemic targeted therapies (i.e. Sorafenib) are the standards for intermediate and advanced-stage HCC, respectively. Nevertheless, in addition to their adverse effects and cost-effectiveness, therapies efficacy are poorly predictable. Hence, there is an urgent unmet clinical need to identify patients that are most likely to respond to TACE or to anti-angiogenic therapy, prior to therapy.

HCC are hypervascular tumors, and accordingly, their diagnosis relies on non-invasive imaging modalities (CT and MRI) able to demonstrate a specific vascular dynamic profile ("wash-in/wash-out"). Although accurate for diagnosis, such imaging techniques are poor for prognosis. Within the tumor micro-environment, the vascular network creates a permissive micro-environment that impacts on progression and treatment response. For instance, recent data demonstrated that fractal analysis of CT perfusion images allowed to cluster patients that were exposed to antiangiogenic therapy (bevacizumab) into short and long survival survivors. Hence, vascular architecture is a promising marker for gauging prognosis and response to therapy in HCC and it can be characterized mathematically via its fractal dimension (FD), which expresses the degree of disorganization of the vascular network.

Up to now, organoid cultures obtained from human HCC only composed of tumor cells have been reported showing this model can recapitulate the histopathology and genetic heterogeneity of the cancer cells. Nevertheless, although encouraging and taking into account tumor heterogeneity between patients, such HCC organoids were cultured in liquid conditions which do not consider i) the mechanical properties and the role played by the tumor micro-environment, and ii) the vascularization.

Given the hypervascular hallmark of HCC, improved biomimetic 3D tumor models ("organoid" system) that include endothelial cells and extracellular matrix whom stiffness match those we quantify in patient, will represent more accurate and advanced tools to better investigate the contribution of tumor heterogeneity between patients and crosstalk between tumor cells and environment in order to evaluate response to therapy.

To mimic human HCC, we co-culture HCC organoids (Fig1, left panel) with human endothelial cells (HUVEC used as a proof of concept) in order to develop vascularized organoids containing functional, i.e. lumenized capillaries generated by auto-assembly of endothelial cells that properly

polarize and deposit their own vascular basement membrane (Fig1, center panel). We then adapt the method of human HCC organoids to biopsy specimen via culturing them in collagen hydrogels of various stiffness that closely mimic mechanical stress in tumor tissue. This is indeed of major importance in order to properly assess, in *in vitro* 3D models, the contributions of physical cues and mechanical regulation of tumor growth and angiogenesis in conditions that most closely mimic endogenous tumor stiffness to evaluate treatments efficacy (Fig1, right panel). Altogether, these 3D models will contribute to more properly evaluate tumor progression and therapeutic treatment *in vitro*.
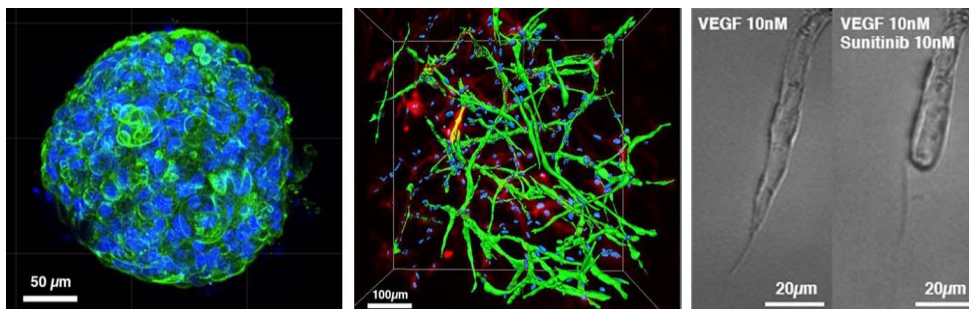

Figure 1: HCC tumoroids (left) and 3D vascular network (center) generated in vitro. These in vitro models are suitable for drug screening: note capillary retraction 48h after Sunitib treatment (right).

Claude Verdier (LIPhy)

Physical approaches to understand cancer cell transmigration

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Cancer cells from primary tumours escape and penetrate the blood flow, then can be transported over long distances. At distant places, they try be pass through the endothelial barrier to reach a new site where a secondary tumour can possibly be formed. In this work we study the mechanisms by which cancer cells achieve this transmigration process.

- AFM is used first to investigate adhesion between cells, in particular the receptors on the endothelium side and the associated ligands [1,2]. We indentified ICAM-1 as a main receptor on the endothelial side and two other possible ligands on the tumour cell side.

- In a second set of experiments, we measure the rheological properties of these cancer cells able to cross the endothelial barrrier. A new microrheology technique allows us to measure local viscoelastic properties (G', G'') and study the effect of a classical substrate stiffness as compared to an endothelium monolayer. The trends observed on classical polyacrylamide gels break down when a biological substrate is used. Invasive cancer cells exhibit a glassy behavior and are less rigid, as shown previously. Then using confocal microscopy we visualize actin remodeling of cancer cells during transmigration, which shows a rapid reorganization of the actin structure leading to the creation of a large protrusion enabling cancer cells to penetrate the barrier. This is in agreement with our previous microrheological findings [3].

- Finally first results on traction force microscopy will be shown to investigate forces developed by cancer cells transmigrating through the endothelium.

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Patricia Davidson (Institut Curie)

Giant nesprins accumulate at the front of nuclei deforming through narrow constrictions

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The nucleus interior is linked to the cytoskeleton through a SUN-nesprin protein complex anchored into the nuclear lamina that spans both nuclear membranes. At the outer surface of the nucleus, nesprins connect to various elements of the cytoskeleton. Alternative splicing of the giant nesprins-1 and 2 (1000 and 800 kDa, resp.) leads to dozens of potential isoforms, but only the full-length isoforms carry both the actin-binding domain and the nucleus anchoring domain.[1] While the cytoskeleton-binding ability of these proteins has been widely reported, the exact roles they play in transmitting mechanical force to the nucleus is still poorly understood. Using CRISPR/Cas9 technology we created a mouse fibroblast cell line in which the endogenous actin-binding domain of giant nesprin-2 is labelled with a green fluorescent protein domain. Intriguingly, these cells display a fluorescent signal predominantly at the nuclear periphery, indicating that the majority of the nesprin-2 actin-binding isoforms expressed are full-length giant nesprins; very little of the other actin-binding isoforms are expressed.

We observed the deformation of these cells during migration through microfluidic devices comprised of narrow constrictions created by closely-spaced pillars.[2] We demonstrate that actinbinding nesprins accumulate at the front of the nucleus as it is squeezed through narrow constrictions. To assess the role of the nuclear lamina, we labelled A-type lamins with a red fluorescent probe using CRISPR/Cas9. Lamins are depleted from the front of the nucleus during deformation, likely due to stretching of the lamina in this area. Nesprin accumulation is thus not due to accumulation of the nuclear lamina and does not recruit lamins. The nesprin accumulation observed is thus likely due to cytoplasmic factors, implicating that the cytoskeleton may be involved in recruiting nesprins to pull the nucleus forward. Preliminary experiments do not indicate that actomyosin is responsible for the nesprin accumulation observed. Experiments to identify the cytoskeletal factors responsible are ongoing. We show here that the predominant isoforms of giant nesprins in fibroblasts are the giant isoforms, and that these accumulate at the front of the nucleus during deformation, likely due to force exertion by the cytoskeleton. Further experiments will identify the cytoskeletal factors involved and the role of nesprins during force exertion to displace the nucleus through obstacles.

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Audrey Prunet

Soft cell confiner development to decipher the impact of mechanical stimuli on cell

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We hypothesize that changes in physical properties, which occur in response to proliferation burst (compressive stress) or increased microenvironment stiffness lead to local changes in mechanical forces compression, which could affectCancer Stem Cells features and resistance to treatment. In such situation, mechanical confinement could last for several days, if not months. So it is important to reproduce this long-term compression, without affecting cell behaviour by other means.

We havedeveloped a hydrogel-based microsystem to study the impact of extended confinement on cancerous cells, without impairing cell survival with hypoxia or nutriment consumption. This biomechanical system with rigidity closer to physiological conditions and enabling efficient medium renewal is compatible with high resolution microscopy and allow to measure dynamic phenotypic and genotypic modifications.Using hematopoietic cells, we were able to show the impact on gene expression upon cell long-compression, with no major impact on cell proliferation.The soft-cell confiner described in this manuscript appears thus as a powerful tool for the growing field of mechano-biology.
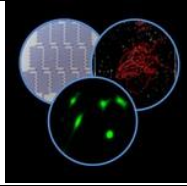
Abstracts of Posters

Ben Meriem Zacchari

Coupling mechanical compression and chemical signaling in tumor

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Pancreatic ductal adenocarcinoma cancer (PDAC) is a particularly aggressive cancer, with a death toll of 99% after 5 years. As of today, there is no efficient treatment or targeted therapy. Cells from this cancer undergo a large series of genetic modifications such KRas activation mutations and p53 silencing which, concomitant with alterations of the interactions between the cells and the stroma, leading to tumor progression. Notably, the rapid cell proliferation in a confined environment, along with the remodeling of the extracellular matrix, leads to growth-induced mechanical compressive stresses that eventually accumulate in PDAC. Although mechanical stresses alter cellular physiology and cancer treatments, we further hypothesize that cancer cells could modulate their response to chemical signals in presence of mechanical stresses. In this context, it is not known if specific mechanical stresses can either promote or restrain tumor progression.

To address these questions, we have developed the first experimental platform based on microfluidics that permits the study of cancer cells in a perfectly controlled mechanical and chemical environment, derived from Holt *et al.* (Methods Cell Biol, 2018). In particular, we can alter the intensity of the mechanical environment of a spatially confined population of cells or determine the mechanical stress the cells actually experience using pressure sensors. Our microfluidic platform also enables the coupling between mechanical and biochemical signals through the dynamic mixing of chemicals of interests such as drugs or cytokines. Moreover, innovative microfabrication approaches including the use of external elements inserted in the device allow us to bring co-culture of stroma with cancer cells and even recover samples of study. We will use this experimental platform to determine how the behavior of a cell's biological response is altered following a variation of both the chemical and mechanical environments. In particular, we will focus on the characterization of how a specific set of mechano-chemical conditions can drive the epithelial-to-mesenchymal transition, a phenomenon critical to the dissemination of PDAC.

Altogether, our work can bridge the biochemical, genetic and mechanical characteristics of cancer cells. Our device can eventually be used to understand cancer progression and explore novel therapeutic strategies incorporating mechanics.
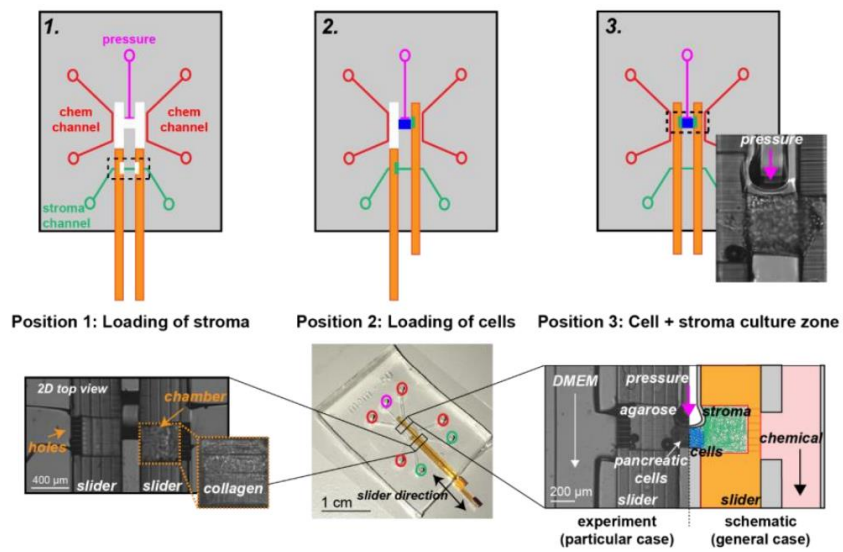
Microfluidic device for the control of mechanical compression, chemical and stromal elements. We manufactured "sliders" with an opening window in which we can flow medium. We initially put two sliders in position 1, to load them with a given "stroma" (ECM - like collagen - and cells of choice) and culture this. Then, sliders are moved to position 2, where cancer cells can be loaded, and finally to position 3 where the two stroma sliders sandwich a cell population. In this example, pancreatic cancer cells are sandwiched between two agarose gels. We can flow any

Bosc Lauriane

Engineering of mini-tumors using biomimetic coatings combined with architectured scaffolds

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Background. 2D cancer cells culture systems on stiff materials such as glass or tissue culture polystyrene (TCPS) have been traditionally used as a tool for the screening of anti-cancerous drugs in pre-clinical trials. However, it is now recognized that cells can perceive their microenvironment, especially its stiffness and biochemical properties and respond to it [1]. For this reason, scientists have begun to develop more complex tumor models that may better recapitulate the biochemical and physical properties of the cancerous tissues [2, 3]. In particular, bioactive signals from the extracellular matrix (ECM) like those initiated by growth factors and chemokines play a significant role in developing drug resistance in cancer cells. Fibronectin (FN) and SDF-1 are a few of the important biomolecules which show a significant effect on the cancer formation and progression.

3D bioprinting enables researchers to study cancer cell behavior in a custom-made 3D environment. Such built scaffolds can accurately be defined as micro- and nano-scale features. Our team previously developed biomimetic films made of hyaluronic acid (HA) and poly(L-Lysine) (PLL) using the layer-by-layer (LbL) self-assembly technique that can be covalently crosslinked and loaded with bioactive proteins [4]. Recently, we used an automated liquid handling robot to deposit the biomimetic films in multiple well cell culture plates in order to do a high-throughput screening of cellular behaviors [5]. The matrix-bound presentation of biomolecules enables to concentrate them very locally and to present them at the basal side of the cells. This presentation mode revealed so far hidden biological phenomena, including an increase in cell adhesion and spreading as well as an increase in biochemical signaling (stronger and long-lasting signal in comparison to the delivery of soluble biomolecules) . Recently our team showed that started the effect of soluble SDF-1α (sSDF-1) and matrix-bound SFD-1α (bSDF-1). Matrix-bound SDF-1 (α SDF-1) increased the cell attachment, spreading and migration in comparison to soluble SDF-1 (sSDF1α). We revealed that there is a crosstalk between the SDF-1a receptor (CXCR4) and the HA receptor (CD44) leading to enhanced Rac1 activation and increased ERK phosphorylation. In the present work, we aim to study at high throughput the behavior of cancer cells on the biomimetic films of controlled stiffness that are loaded with bioactive proteins. Ultimately, by combining a 3D architectured material with the biomimetic film as a surface coating, we aim to recreate in vitro a mini-tumor.

Materials and methods
Polyelectrolyte multilayer film. Polyethyleneimine (PEI; 181978, Sigma-Aldrich), Poly(L-Lysine) (PLL; P2636, Sigma-Aldrich). Hyaluronic Acid (HA; Lifecore, USA) were dissolved in Hepes-NaCl buffer (20 mM Hepes at pH 7.4, 0.15 M NaCl) at 5 mg/mL, 0.5 mg/mL and 0.5 mg/mL respectively. The films were built in 96 well using an automated liquid handling robot as described recently [5]. Films were crosslinked to different levels (EDC30, low crosslinking and EDC70, high crosslinking) by varying the amount of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, E7750, Sigma Aldrich) in combination with N-hydrosulfosuccinimide (sulfo-NHS; 106627-54-7, Chemrio). The crosslinked films were post-loaded with the extracellular matrix protein (ECM) fibronectin (bFN)**.** Protein loading was carried out based on the previous work[4]. Different concentration of fibronectin (5 µg/ml to 20 µl/mL) and were

dissolved in 1 mM HCl. 50 µL of the protein solution was added to each well and incubated at 37°C for 2h. After incubation, the wells are washed with the Hepes/NaCl buffer for several times to remove unbound proteins. Before loading the cells, the plates were sterilized using UV light for 20min.

Cell culture. MDA-MB-231 (Human epithelial breast cancer cells) cell line was purchased from ATCC (ATCC® HTB-26™). DMEM medium contains 10% fetal bovine serum (FBS, PAA Laboratories) and 1% antibiotics (penicillin G and streptomycin) was used for cell culture.

Fluorescence imaging of the cells. MDA-MB-231 cells were seeded at 12 500 cells/cm² in the 96 well plates containing the different biomimetic films. The cells were cultured at 37°C with 5% CO_2. After incubation, the cells were fixed and labeled with rhodamine-phalloïdin for the actin cytoskeleton (1:800, Sigma Aldrich) and DAPI for the nucleus (1:1000, Sigma Aldrich) in order to image the cells. Fully automated image acquisition was made using a Zeiss Axio Observer 7 with 20X plan apochromatic objective. Each microwell was imaged at 5 positions and in each position a 4-tile image was taken. Cell adhesion and spreading were quantified using a custom-made ImageJ macro [5].

Results. We successfully prepared the polyelectrolyte films of the different crosslinking level (EDC30, EDC70) using an automated liquid handling robot. The thickness of the films was uniform inside each well and between different microwells [5]. The number of adherent cells and the cell spreading area were assessed by staining the nucleus and actin cytoskeleton. We observed that the number of cells adhered to the EDC70 films was similar to that on the tissue culture plates. In contrast, only a few cells adhered on the films crosslinked at a low level (EDC30), due to their lower stiffness. Cell spreading was also higher on the stiffer films than on, the softer ones. The effect of bFN on the MDA-MB-231 cell adhesion was studied by culturing the cells on films presenting increasing concentrations of bFN. The cell number and cell spreading area increased with the concentration of bFN, cells being sensitive to even small concentrations of FN (5 µg/mL). Finally, we designed 3D architectured scaffolds in a synthetic polymer using two-photon polymerization with the ultimate goal of providing a 3D microenvironment and a biomimetic film to the cancer cells.

Conclusions.
Our results show that the biomimetic films made of (PLL/HA) of different crosslinking level can be fabricated reproducibly in order to be used for the high throughput screening of cancer cell processes. Here, we focused our study on early adhesion and spreading of human breast cancer cells. Cell adhesion and spreading depended on both the film stiffness and the amount of adhesive extracellular matrix protein that was provided via the film. In our future work, we will combine 3D architectured scaffolds with the biomimetic films in order to engineer in vitro a mini-tumoral niche.

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Erwan Eriau

Single-cell, long-term optogenetic control of gene expression

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Une grande partie des expériences de biologie consistent à observer l'effet d'une perturbation sur un organisme ou une cellule. Les questions biologiques auxquelles nous pouvons espérer répondre sont limitées notamment par les perturbations que nous sommes en mesure appliquer, et notamment:

1) La constance de cette perturbation dans un environnement fluctuant, malgré la variabilité cellulaire

2) La variation de cette perturbation à travers le temps et les individus d'une même population

3) L'application différenciée de la perturbation en fonction de l'état cellulaire

Nous proposons un appareillage expérimental adressant ces enjeux en permettant la régulation en temps-réel et à long-terme du niveau d'expression d'un ou plusieurs gènes dans plusieurs cellules individuellement. Cet appareillage consiste en l'assemblage de trois techniques éprouvées:

1) Une chambre microfluidique, qui permet la culture des cellules optogénétiques dans des conditions bien définies et aisément modifiables

2) Un microscope, qui assure aussi bien l'observation des cellules que leur stimulation individuelle, grâce à un DMD.

3) Un ordinateur, qui assure la précision et la durabilité du contrôle, grâce à un modèle interne de la réponse - et permettra à terme une délocalisation d'une partie de la décision cellulaire in silicco.

Nous appliquons cette technique à la voie Hog de réponse au stress osmotique de la levure

Goodarzi Saba

Use of hydrogel-based microsystems for high-throughput quantification of Cy5-conjugated AGuIX© nanoparticles penetration within multicellular tumor spheroids (MCTS)

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Gadolinium-based nanoparticles (Aguix®) have been proved as an efficient tool for theranostic applications including diagnosis of diseases, monitoring of antitumoral therapy, radiotherapy and drug delivery to tumors. Tumors in the body are 3D structures composed of cells aggregates and blood vessels network, therefore the penetration of nanoparticles, inside this 3D structures, is complicated and nanoparticles are mostly uptaken at the tumor surface.

Limited predictive power of conventional *in vitro* experiments to test anti-cancer therapeutic strategies encouraged us to use MCTS as a 3D *in vitro* model for tumors which is able to mimic tumor micro-environment.

In this study we used colorectal cancer cell spheroids as a 3D in vitro model to evaluate the penetration of nanoparticles inside tumors. For this purpose, MCTS of HCT116 cell line are prepared by using a hydrogel-base microsystem. AGuIX® nanoparticles functionalized with Cy5 are used to quantify their penetration inside MCTS via fluorescence confocal microscopy. We found that AGUIX® nanoparticles penetration is both dependent on concentration and incubation time. As our hydrogel-based microsystems is compatible with in situ immunostaining and clarification techniques, we were also able to quantify nanoparticles localization within cells and the impact on cell proliferation in the entire volume of this 3D structures.

Combining our hydrogel-based microsystems with confocal microscopy and clarification techniques appears thus as a valuable tools to quantify cell-nanoparticles interactions within MCTS.

Lecot Solène

A simulation study of antibody/antigen interaction: a tool for Circulating Tumor Cell characterization

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Circulating tumor cells (CTCs) are tumor cells that separate from the tumor and join the bloodstream. They play an important role in metastasis dissemination and are characteristic of the tumor from which they shed. To study the metastatic power of these CTCs and to test their chemosensitivity towards new molecules, it is necessary to capture them alive. CTC expose specific proteins (antigens) on their membrane which can be specifically targeted by antibodies (Ab). Thus the capture of CTC could be triggered by Ab immobilization on surfaces through antigen / antibody interactions. To this aim, antibody against CTC membrane antigen will be immobilized on a functionalized SiO2 surface allowing CTC isolation and further characterization. The SiO2 surface will be functionalized by a silane monolayer as the team has expertise in silanization [1, 2] and previously performed immobilization of different proteins on these silanized surfaces [3].

Our first objective is to study, at the molecular scale, the relationship between the way of immobilization of the antibodies on the silanized surface and their biological activity, i.e. their ability to interact with their antigens. We will combine Molecular Dynamics (MD) simulations and Atomic Force Microscopy (AFM) in the mode Single Molecule Force Spectroscopy (SMFS) experiments.

SMFS-AFM allows to quantify the strength of the antibody-antigen interaction at the molecular scale. Some previous experiments have been done in the lab on protein/glycocluster interactions [4]. MD simulations enable to model the system and to investigate interactions at the atomistic scale [5].

In order to validate our methodology, we have chosen a very well-known model system, the streptavidin – biotin complex. Indeed, some experimental and simulation results have already been established on this complex [6]. However, to our knowledge, the effects of a silane layer on the streptavidin immobilization and on its interaction with biotin have never been explored. We develop a MD simulation system to investigate such interactions (GROMACS – force field OPLS).

First results obtained indicate an effect of silane density on the configuration of streptavidin on the surface.

These results will be completed by both steered MD simulations (AFM simulations – pulling on the biotin to detach it from streptavidin such as in a force-displacement curve) and experimental AFM to characterize the energy landscape between streptavidin and biotin. Our model will then be extended to the EpCAM / anti-EpCAM system, which is the antigen/ antibody system chosen for the capture of breast cancer CTCs.

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Lipp Clémentine

Design of a microfluidic chip for the formation of cell pairs using dielectrophoretic manipulation and trapping

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Adoptive cell therapy (ACT) is a promising immunotherapy approach as it can tackle tumors with specific sets of genomic alterations and mutations that would be insensitive to generic immunotherapy. The process consists first in harvesting T-Lymphocytes (T-cells) from a patient and then in recognizing and isolating the 0.1 % of the total population with tumor specific activity. The active T-cells are then expanded *in-vitro* before being reintroduced in the patient to actively direct an immune response to fight the tumor. Isolating the 0.1 % of active cells represents the major challenge of the process as the concentration is below current isolation technique capabilities.

The project aims at developing a high throughput microfluidic device able to bring T-cells in close contact with tumor cells to determine their affinity and isolate the reactive T-cells in a separate channel. The use of vertical microfluidic traps is foreseen for the immobilization of the tumor cells at specific locations, whereas dielectrophoresis (DEP) will be used to direct the T-cells toward the location of the different tumor cells, where they will be maintained against the flow by dielectrophoretic traps during the interaction time as presented in Figure 1. After the DEP trapping is released, the T-cells without tumor affinity are expected to be dragged by the flow whereas the cells presenting an affinity for the tumor cells are expected to remain attached to them.
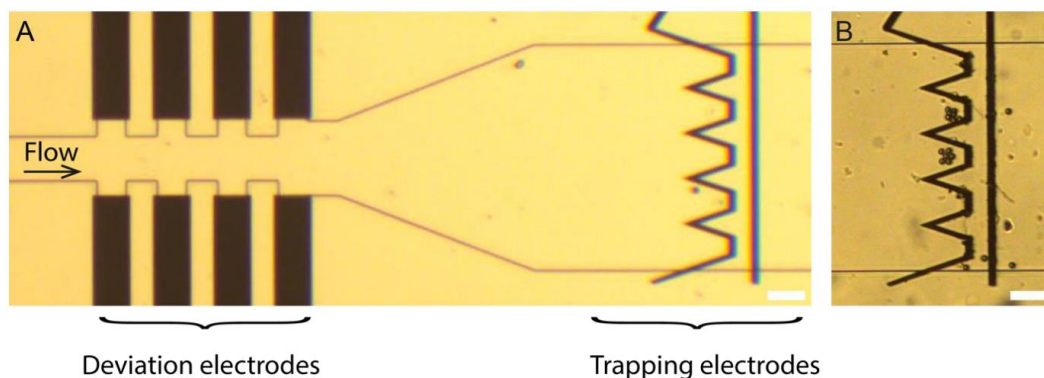

Figure 1 A) Electrodes design for the deviation and trapping by DEP B) Beads maintained against the flow by the DEP traps. Scalebar 50 μm.

This device is foreseen for cancer treatment as well as for the study of single cell-cell interactions with applications in fundamental biology research.

Manssouri Hanane

Study of cell migration and nucleus stiffness using microfluidic devices in Triple-Negative Breast Cancer cell lines

Institut Curie (France)

The presence of cancerous cells in tissues other than the primary tumour (called metastasis) is a poor prognostic indicator. To migrate through tissues, cancer cells have to leave the primary tumor and reach circulatory systems. This implies that cancer cells have to cross boundaries and narrow constrictions exerting forces on their stiff and large nucleus. Thus one of promising option to reduce cancer-related death is to target the mechanisms that lead to metastasis: migration and invasion. Triple-Negative Breast Cancer (TNBC) cell lines are a subset of breast cancer that is not responsive to conventional treatments (chemotherapies) and which presents high rates of early distant metastatic events. Here we proposed to study the migration ability and nucleus deformability through narrow constrictions of TNBC cells derived from metastatic sites, compared to primary tumor cells. We studied a panel of seven cell lines established from TNBC primary tumours, two cell lines isolated at Curie from peripheral lymph nodes around a TNBC tumour, and five cell lines established from pleural effusions. Preliminary results using microfluidic migration devices indicate that, on average, the cells derived from metastatic sites studied are able to deform their nucleus more efficiently through 3D tight spaces than the primary tumor cell lines. To determine whether this effect is related to the nucleus stiffness we are studying the deformability of our panel of TNBC cell lines using microfluidic devices that mimic micropipette aspiration. In parallel, to understand whether the nucleus deformability is due to a more efficient transmission of forces to the nucleus, studies of proteins linking the nucleus to the cytoskeleton are underway. From this study, we expect to determine whether cells derived from metastatic sites are primed to undergo migration through tissues.

Vezy Cyrille

Non Radiative Excitation Fluorescence Microscopy: a new method for studying membrane adhesion at the nanoscale

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Non-radiative Excitation Fluorescence Microscopy (NEFM) is a promising technique allowing the observation of biological samples beyond the diffraction limit. By coating a substrate with a homogenous monolayer of quantum dots (QDs), Förster Resonance Energy Transfer (FRET) could be achieved from QDs (donors) to dye molecules located in the sample (acceptors). Therefore, the excitation depth of the sample is then given by the Förster radius, which corresponds to few nanometers above the surface. Here, we present this original method to probe the adhesion of Giant Unilamellar Vesicles (GUVs), negatively charged), in strong interaction with a positively charged surface (QDs layer is coated with Poly-L-Lysine). Distances between the surface and GUVs are lower than 5 nm. We used the QDs-quenching level to calculate and map the absolute distance between the membrane and the surface with a nanometer resolution. By tuning the electrostatic interactions between the surface and the membrane, we have been able to measure a displacement of about 1 nm of the lipid membrane height [1].

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Yang Zihua

MD Simulations of silanized surfaces for the development of cancer diagnosis micro-array

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Surface functionalization for biomarkers immobilization is a key concern for the development of early cancer diagnosis system. Silane molecules have been widely reported for the immobilization of protein in diagnosis microsystems [1].In our lab, the effect of the nature of the silane on the recognition process was established [2]. However, the understanding of the interactions between proteins and silanes on solid surface remain unraveled due to a lack of in operando characterization tools. Molecular dynamic simulation of silane layers have been only recently reported [3].

Herein, we report on MD simulations of different silanized surfaces with different conditions, using GROMACS software. Starting from the OPLS force field file of the FDTS silane molecule ($C_9H_7O_3F_7$) described by [3], we have built the force field of three other silanes molecules ($C_{21}H_{46}OSi$, $C_6H_{16}OSi$, $C_7H_{19}NOSi$) at 3 different pH (2, 7, 12). We have organized silanes molecules into monolayer with 3 different densities on amorphous SiO_2 surface, in explicit water. The maximal density corresponds to the maximal experimental density, 3 silanes/nm^2. To avoid surface curvature due to periodic boundary conditions a wall of Lennard-Jones particles were added in each simulation box, as recently proposed by [4]. Our results indicate the influence of pH, silane functional groups, silane alkyl chain length, and density on surface tension, on water contact angle and on the clustering of the free extremity of the silane molecules.

These results were validated through experimental characterizations. SiO_2 surfaces were silanized with the same silane molecules as in simulations, by a classical experimental protocol available at INL. The density of silane was measured by XPS and used in our simulations. The water contact angle was measured and surface tensions were evaluated. Results are compared with simulations.

So we have built reliable MD simulations systems to go further and simulate interactions of proteins with different silane layers on SiO_2 surface at different pH. Such simulations will allow to understand interactions between silane layer and proteins, and to optimize surface functionalization for biomarkers immobilization for cancer diagnosis.

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Irinka Séraudie

COMBOREIN: The pre-clinical trial assessing the susceptibility of patients with clear cell Renal Cell Carcinoma to drug response

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Clear cell renal cell carcinoma (ccRCC) is the third type of urologic cancer. At time of diagnosis, 30% of cases are metastatic with no effect of chemotherapy or radiotherapy. Current targeted therapies lead to a high rate of relapse and resistance after a short term response. Thus, the development of new treatments is challenging scientists and necessitates adapted models to test drug response. We previously developed two drug combinations that target respectively ATM + CK2 or PI3K + Src kinases (1,2). We are currently challenging these combinations toward clinically used drugs like Sunitinib, Pazopanib or Temsirolimus, comparing different 3D cultures models of ccRCC as spheroids or ex vivo tissue slice culture. Here, we show the feasibility and the advantage of human ccRCC tissue slice culture as a preclinical model.

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Marjorie Dufaud, Coralie Durieux

Development of new in vitro tumor models using 3D bioprinting

Application to breast and lung cancers

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Key words: 3D bioprinting, Cancer, in vitro 3D model, Microenvironment, Drug screening

With the aging of populations, it is to be expected a growing number of health concerns affecting morbidity and well-being through life. Among diverse age-related diseases, we can cite cancers which are the leading cause of death in France today (28.7%), right before cardiovascular diseases (25%). Indeed, 355 000 new cancer cases are detected each year (200 000 for men, 155 000 for women). Among the diversity of cancer types, breast and lung cancers are within the top 3 of the most frequent cancers developed.

2D cultures and animal models are commonly used in oncology research to study pathogenesis mechanisms or to predict the effectiveness and toxicity of a drug.[1] However, these models fail to mimic the complex process of human pathophysiology. In 2D cultures, cells grow as a monolayer under simplified and non-physiological conditions that do not recapitulate the complex interactions and spatial organization of cells in a tumor microenvironment.[1] Animal models reproduce a 3D architecture closer to human physiology, but still show many limitations especially due to inter-species biological divergences. Besides, animal models imply ethical considerations regarding the discomfort and pain of animals during experimentation.[1] To overcome these limitations, new cellular models closer to the pathophysiological conditions and tumor microenvironment found in human must be developed. *In vitro* 3D models, mainly characterized by the formation of spheroids that are considered as small tumors, have been developed to improve *in vitro* assays. But here again, the limits of the models prevent the direct transposition of the observed results to humans.[1]

In this context, 3D bioprinting can be used to introduce cells into extracellular matrix materials and form a more complete *in vitro* 3D model.[2,3] In our work, breast (MDA-MB-231) or lung cancer cells (A549) were printed in a bio-ink composed of a mix of gelatin, alginate and fibrinogen at 28°C and 21°C respectively. Other cells, such as fibroblasts, were added to tune the breast cancer model. Microscopic observations of the printed constructs show the

ability of both cell types to form spheroids within the hydrogel, allowing spheroid culture within a controlled environment. In the next steps, diverse paths (addition of immune cells, coupling to microfluidic, etc.) could be studied to make these models more complex and more realistic for deeper analysis, both in terms of pathologies studying and drug screening.[4]

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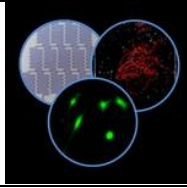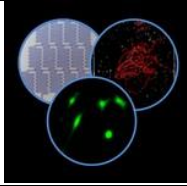
Cancer Cells-on-Chip 2

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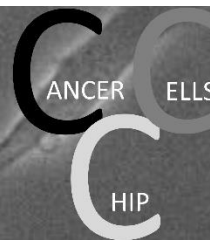audrey.ziverec@inserm.fr   Centre de Recherche en Cancérologie de Lyon

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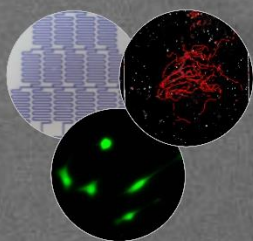
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