Sujet thèse / PhD subject 2025

Titre Thèse	Micro-actionneurs biomimétiques pour la capture de cellules tumorales circulantes en dispositif microfluidiques	
PhD Title	Biomimetic micro-actuators for CTC smart trapping using mictofluidic devices	
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Projet phare principal		
Demande de fléchage IEMN ? (Energie / Nanocaractérisation / Technologies Neuromorphiques)	Oui ./ Non : **NON** Flagship choisi :	
Demande de labellisation Université de Lille (GREAL, labellisée)	Oui / Non : **OUI** Label : **Chaire WILL**	
Financement acquis Oui ☐ Non ☒ Partiel ☐	Si acquis (total ou partiel), préciser : (contrat, organisme, Université étrangère, , ….) :	
Financement demandé	Contrat Doctoral Etablissement	ULille ☒ Centrale Lille ☒ JUNIA ☒
	Région ou Autre ☐ Préciser :	Co financement (Préciser l'origine, demande en cours, et si acquis ou pas) :

A. Résumé / Abstract :

Dédié à l'affichage sur le site de l'IEMN Typiquement 5000 charactères maximum)

Circulating tumor cells (CTCs) are cancerous cells that detach from tumors and enter the bloodstream, driving cancer spread. Accurate enumeration of single CTCs and clusters in liquid biopsies is vital for understanding tumor biology and improving prognoses. CTC clusters, associated with worse survival rates, vary in frequency and size across tumor types, making their analysis valuable for diagnostics, but this diversity and their dynamic behavior make their isolation and a real challenge with actual technology. In particular, the epithelial-mesenchymal transition (EMT), a process enhancing cell motility and invasiveness, complicates CTC isolation due to their heterogeneity and mixed epithelial-mesenchymal phenotypes, which challenge techniques relying on epithelial markers like EpCAM.

The overall goal of this project is the development of synthetic cells (SynC) for capture and analysis of circulating tumor cells (CTC) within a liquid biopsy sample. The SynC exhibits an artificial external receptor (antibody) that binds to one or more CTC (CD18, EpCAM). The voluminous complex SynC-CTC is then easier to separate from the rest of the sample by filtration, and can be retrieve by pipetting.

From there, the refined sample is loaded on a microfluidic device developed in the laboratory to isolate SynC-CTC. This action triggers a molecular program embedded in the artificial cell for release of the SynC or in-situ analysis of the CTC content (fusion of membrane and detection of molecular targets). Altogether, this system will facilitate diagnosis and explore the cellular biology of CTC with minimal impact on their survival rate.

B. Compléments

Pour évaluation en interne à l'IEMN. Sont concernées uniquement les demandes de thèse Région, Labellisée Université de Lille et de fléchage IEMN). 3 pages maximum.

1. Le sujet de recherche choisi et son contexte scientifique et économique :

Circulating tumor cells (CTCs) are cancerous cells that break away from primary or metastatic tumors and enter the bloodstream, contributing to the spread of cancer to distant organs. Accurate enumeration of single CTCs and the size distribution of CTC clusters in a liquid biopsy is crucial for understanding tumor biology and providing more precise cancer prognoses (Masuda et al., Molecular Oncology, 2016). However, it is increasingly clear that CTC clusters—groups of cancer cells found in circulationhave a significant clinical impact, being associated with poorer survival rates in patients (Aceto et al., Cell, 2014). The distinct frequency and size distribution of CTC clusters across different tumor types suggest that detailed analysis of these clusters is valuable for clinical diagnostics and prognostics. A key process influencing the behavior and characteristics of CTCs is epithelial-mesenchymal transition (EMT), during which cancer cells lose their epithelial characteristics (such as cell-cell adhesion) and gain mesenchymal traits that enhance their motility and invasiveness. This dynamic process is crucial for metastasis, making CTCs highly heterogeneous and difficult to isolate. Some CTCs may exhibit mixed epithelial and mesenchymal phenotypes, further complicating their detection, as many isolation techniques rely on epithelial markers like EpCAM.

Isolating CTCs from whole blood remains challenging due to their low abundance (~10 cells per mL of whole blood) and heterogeneity of their profiles. Biophysical approaches, which exploit differences in size, deformability, and electrical properties, offer label-free alternatives but often fail to differentiate between single cells and clusters due to device size limitations. On the other hand, immuno-chemical methods involve the use of antibodies that target specific surface markers, such as EpCAM, which are typically expressed on epithelial CTCs. While these techniques can be highly specific, they may miss CTCs that have undergone EMT and lost epithelial markers or those that do not express the targeted antigens. Additionally, the use of antibodies and labeling can potentially alter the native state of the cells, affecting downstream analysis (Figure 1).

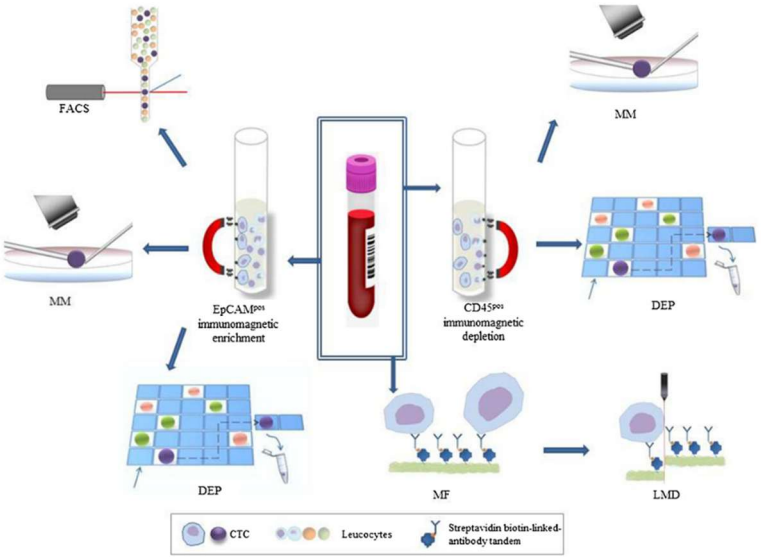

Figure 1: main combinatorial methods for CTC isolation (Mansilla et al., 2018)

Combined methods for isolating CTCs often employ a dual approach that integrates both labeling techniques and physical separation methods, such as magnetic beads or microfluidic devices (Park et al., Lab on Chip, 2022). These methods leverage the specificity of antibodies against known tumor markers (e.g., EpCAM) alongside physical properties like size and density to enhance the efficiency of CTC capture. While this combined approach can improve isolation rates, it is not without limitations.

One significant drawback is that the reliance on epithelial markers may lead to the exclusion of CTCs that have undergone EMT, resulting in a loss of these markers and a shift towards a more mesenchymal phenotype. This can create a population of CTCs that are undetectable by conventional immunoaffinity techniques, potentially skewing analysis results and underrepresenting the tumor heterogeneity. Furthermore, the introduction of antibodies and the mechanical forces involved in physical separation may alter the native state of CTCs, influencing their viability and functionality post-isolation. Therefore, while combined methods show promise, there is a critical need for the development of novel label-free techniques that maintain the integrity and diversity of CTC populations, thereby enabling more accurate downstream analysis.

Synthetic cells (SynC), often composed of lipid bilayers or proteinosomes, are engineered compartments designed to mimic natural cellular functions. These compartments offer programmable properties, allowing researchers to integrate synthetic genetic circuits and cell-free protein synthesis systems to produce biomolecules in a controlled environment. This adaptability is key for applications in precision health, where synthetic cells can be used for real-time biosensing (Garamella et al., ACS Synthetic Biology, 2019), therapeutic delivery (Diltemiz et al., Materials Chemistry Frontiers, 2021), and cellular communication (Lentini et al., Nature Communications, 2018). For example, their ability to respond to environmental signals and produce specific outputs opens up possibilities in areas such as tissue regeneration, immune modulation, and treatment of complex diseases. Moreover, SynCs' programmability allows them to interact with biological systems in ways that traditional tools cannot. They can be engineered to sense particular molecular environments or changes, providing opportunities for early diagnosis of diseases. These cells could detect biomarkers or pathological changes within the body and trigger a therapeutic response, thereby offering real-time, targeted diagnostic tools that surpass conventional

techniques in precision and adaptability. The potential to monitor multiple biomarkers or conditions simultaneously positions synthetic cells as a promising technology for future diagnostics, with far-reaching implications for personalized medicine.

2. *L'état du sujet dans le laboratoire d'accueil.*

The student will benefit from the resources and expertise of IEMN in microfabrication (CMNF: lithography and bio-microfluidics) for the design, optimization, and production of microfluidic devices dedicated to vesicle formation. A. Baccouche, a CNRS Research Scientist recruited this year, is developing organic BioMEMS (artificial cells) and brings 10 years of expertise in synthetic biology (in-vitro reconstruction of biosynthetic pathways), microfluidics, and DNA nanotechnology, which are at the core of this project. Furthermore, improvements to the instrument suite and the diversification of molecular biology offerings achieved through the CPER TECSANTE program will support the PhD student in the comprehensive characterization of these new molecular tools. This interdisciplinary and innovative project will foster a research dynamic at IEMN and help establish or strengthen connections between the institute's historical themes and new inspirations/applications through novel collaborations).

SHK is a lecturer at the Institute of Industrial Science, University of Tokyo whose work focuses on the capture of CTCs using microfluidic methods. SHK is an expert in microfabrication and bioMEMS, and has partnerships with both academic and industries (Sharp, NTT, Canon Medical…). The student will take this advantage to test the SynC in an international environment with direct patient samples through diverse SHK collaborations in Japan.

3. *Les objectifs visés, les résultats escomptés.*

The main objective of the PhD project is the conception and use of SynC to trap CTCs within a microfluidic device. We will need to leverage 2 technical challenges there : (i) producing thanks to a microfluidic device micro-actuators (SynC) able to detect and trap CTC within a biopsy sample and (ii) retrieve the SynC-CTC complexes within a diagnosis chip.

The first phase involves the creation of active SynC using microfluidic technology, building on the method by Suzuki et al. The SynC will be designed to recognize CTC surface receptors by functionalizing the SynC with antibodies (e.g., EpCAM) while embedding a fluorogenic peptide, such as GFP, within the synthetic cells. Unlike traditional beads, the density and deformability of SynC allows them to bind to CTCs faster and more efficiently. This deformability, combined with their ability to encapsulate fluorescent agents such as GFP and target specific CTC surface proteins, makes SynC an excellent aggregating agent, addressing the issue of the scarcity of CTCs in blood samples.

The second stage focuses on the proof of concept for isolating CTCs. When introduced into a liquid biopsy, SynC binds to the CTCs, making the SynC-CTC complex larger and easier to isolate through filtration. The fluorescent labeling on the SynC also makes the CTCs more identifiable. Unlike conventional filtration methods that discard larger particles, this approach leverages the increased size and weight of the SynC-CTC complex for easier separation. The SynC also facilitates anchoring to the microfluidic chip surface through thiol-rich moieties, streamlining the process.

4. *Le programme de travail avec les livrables et l'échéancier prévisionnel.*

- **Literature Review (Months 1-3):** Conduct a comprehensive review of existing research on synthetic cells (SynC), microfluidics, and CTC isolation methods. Compile and analyze key methodologies, materials, and technologies from relevant publications. Refine project goals and experimental setup (simulations). Identify and source necessary materials, including antibodies, peptides, and microfluidic devices.
- **Preliminary Training and Setup (Months 3-6):** Acquire technical skills in microfluidic technology and fluorescence microscopy. Set up laboratory protocols and initial microfluidic chip designs for SynC synthesis.
- **Development and Testing of SynC (Months 7-12):** Fabricate SynC prototypes using microfluidics based on the Suzuki et al. methodology. Optimize design to fit features of SynC. Functionalize SynC with antibodies (e.g., EpCAM) and embed fluorogenic peptides (e.g., GFP). Optimize SynC physical properties (density, deformability) for improved binding to CTCs.
- **Optimization and Validation (Months 13-18):** Test SynC binding efficiency to CTClike cells in controlled conditions. Optimize fluorescence signal for identification and tracking. Iterate on SynC designs based on experimental feedback.
- **Integration with Microfluidic Systems (Months 19-22):** Design and fabricate microfluidic chips for SynCCTC filtration and isolation. Functionalize chip surfaces with thiol-rich moieties to enable SynC anchoring.
- **Validation in Simulated and real Environments (Months 23-29):** Simulate liquid biopsy conditions and introduce SynC to isolate CTCs. Evaluate filtration efficiency, fluorescence detection, and SynC anchoring performance. Then, validate SynC-CTC capture efficiency, fluorescence tracking, and filtration on clinical samples.
- **Thesis Writing and Defense Preparation (Months 29-36**): Write and compile the PhD thesis. Prepare for the defense presentation with key findings and experimental insights.

5. *Les collaborations prévues*

This PhD project lays on the framework of international WILL chairs of the University of Lille. The first consequence is that this project is a collaboration between the young co-PI and a PI abroad. The candidate will develop the SynC in IEMN (AB) and will apply them at the University of Tokyo (SK) where the platform for manipulation of patient sample is available.

Within the course of the project, the student will interact locally with Alexis Vlandas and Chann Lagadec who are directly linked to the project, as well as other partners of AB on the SynC production platform.

6. *Une liste de 10 publications maximum portant directement sur le sujet en soulignant celles du laboratoire.*

Park J., Park C., Sugitani Y., Fujii T., **Kim S.H.**; An electroactive microwell array device to realize simultaneous trapping of single cancer cells and clusters, Lab Chip, 2022, 22, 3000

Baccouche A., Okumura S., Sieskind R. Henry E., Aubert-Kato N., Bredeche N., Bartolo J.F., Taly V, Rondelez Y., Fujii T, Genot A.J., Massively parallel and multiparameter titration of biochemical assays with droplet microfluidics, Nature Protocols volume 12, pages1912–1932 (2017).

Nader S., **Baccouche A**., Connolly F., Abou-Ghanem M., Styler S.A., Lewis J.D., Pink D., Mansy S.S., Model Atmospheric Aerosols Convert to Vesicles upon Entry into Aqueous Solution, ACS Earth Space Chemistry. 2023, 7, 1, 252–259

S. Okumura S., Gines G., Lobato-Dauzier N., **Baccouche A.**, Deteix R., Fujii T., Rondelez Y., Genot A. J., Nonlinear decision-making with enzymatic neural networks, Nature volume 610, pages496–501 (2022)

Kuruma Y., Ueda T., The PURE system for the cell-free synthesis of membrane proteins, Nature Protocols volume 10, pages1328–1344 (2015).

Ushiyama R., Koiwai K., Suzuki H., Plug-and-play microfluidic production of monodisperse giant unilamellar vesicles using droplet transfer across Water–Oil interface, Sensors and Actuators: B. Chemical 355 (2022) 131281.

Boyd M.A., Kamat N. P., Designing Artificial Cells towards a New Generation of Biosensors, Trends in Biotechnology, 39 (9), 2021, 927-939