Title of the PhD project:

Development and characterization of bio-inspired scaffolds with advanced electromechanical properties for cardiac tissue engineering.

PhD Supervisor

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Team leader (*if different*) *:* AGBULUT Onnik & LI Zhenlin
Doctoral School : ED394 « Ecole Doctorale Physiologie, Physiopathologie et Thérapeutique ».

Overview of the scientific projects of the team

Our main interest is to understand the pathophysiological mechanisms of cardiovascular diseases and ageing, to identify and develop new therapeutic strategies. We address these issues through research on stem cells, the development of bio-inspired biomaterials, tissue engineering, 3D cell culture, the development of cellular and animal models. More precisely, our research is centered on the mechanisms of cardiovascular remodeling in age- and disease-related heart failure devoting attention to cytoskeleton organization and mechanotransduction pathways.

Main publications since January 1er, 2016

- Langlois B., Belozertseva E., Parlakian A., Bourhim M., Gao-Li J., Blanc J., Tian L., Coletti D., Labat C., Ramdame-Cherrif Z., Challande P., Regnault V., Lacolley P. and **Li Z** (2017). Vimentin knock out results in increased expression of sub-endothelial basement membrane components and carotid stiffness in mice. *Sci. Rep.* **7,** 11628. doi: 10.1038/s41598-017-12024-z.
- Diguet N., Trammell S.A.J, Tannous C., Deloux R., Piquereau J., Mougenot N., Gouge A., Gressette M., Manoury B., Blanc J., Breton M., Decaux J.F., Lavery G., Baczkó I., Zoll J., Garnier A., **Li Z.**, Brenner C. and Mericskay M. (2018). Nicotinamide riboside preserves cardiac function in a mouse model of dilated cardiomyopathy. *Circulation.* **137,** 2256-2273.
- Deloux R., Tannous C., Ferry A., **Li Z.** and Mericskay M. (2018). Aged Nicotinamide Riboside Kinase 2 Deficient Mice Present an Altered Response to Endurance Exercise Training. *Front*

Physiol. **9,** 1290. doi: 10.3389/fphys.2018.01290.
- Angelini A., Gorey M.A., Dumont F., Mougenot N., Chatzifrangkeskou M., Muchir A., **Li Z.*,** Mericskay M and Decaux JF. (2020). Cardioprotective effects of α-cardiac actin on oxidative stress in a dilated cardiomyopathy model. *FASEB J.* **34,** 2987-3005. ***corresponding author.
- Ferry A., Mésseant J., Parlakian A., Lemaitre M., Roy P., Delacroix C., Lilienbaum A., Hovhannisyan Y., Furling D., Klein A, **Li Z.** and Agbulut O. (2020). Desmin prevents muscle wasting, exaggerated weakness and fragility, and fatigue in dystrophic Mdx mouse. *J Physiol* **598,** 3667-3689.

PhD Co-Supervisor

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Laboratory: UMR 8587
Title of the team : MPI
Team leader *(if different) :* PELTA Juan
Doctoral School: 2MIB, Univ. Paris-Saclay

Overview of the scientific projects of the team

The Lambe is a leader for dynamics in confined medium, electrical detection of protein conformations, and size and sequence identification of peptides through nanopores and data analysis. The Lab is expert on surface functionalization and supramolecular chemistry manufacture. The strong background is also on mechanical properties of membranes, living cell, protein, cell biology and AFM. We have national (ENS, College de France, C2N, Institut Pasteur, Institut Curie, Hopital Lariboisière…) and international collaborations (EPFL, Univ. Notre Dame, Univ Freiburg, Univ. Illinois…).

Main publications since January 1er, 2016

- Smolyakov G., Thiebot B., Campillo C., **Labdi S.**, Severac C., Pelta J. and Dague E. (2016). Elasticity, adhesion, and tether extrusion on breast cancer cells provide a signature of their invasive potential. *ACS applied materials & interfaces* **8**, 27426.
- Lamour G., Allard A., Pelta J., **Labdi S.**, Lenz M. and Campillo C. (2020). Mapping and modeling the nanomechanics of bare and protein coated lipid nanotubes. *Physical Review* **X 10**, 011031.

Doctoral Project

Title: Development and characterization of bio-inspired scaffolds with advanced electromechanical properties for cardiac tissue engineering.

Abstract:

This PhD project aims to produce 3D bio-inspired scaffolds for cardiac tissue engineering. We showed that nanofibrous scaffold mimicking the fibrillar structure of extracellular matrix can be produced by electrospinning. Specifically, our goal is to develop electrospun scaffolds based on piezoelectric nanofibers to obtain bio-inspired materials with improved electro-mechanical properties to explore cardiomyocyte mechano-sensitivity. Indeed, the heart is a mechanically active organ able to sense and respond to its environment. Cardiac cells transduce mechanical forces into electric currents, which modulate heart activity. However, the mechanisms through which forces are converted into electric signals and vice-versa are not fully understood. Through this project, we will investigate the electromechanical forces that modulate cell behavior in the cardiac muscle. Our objective is to untangle the complex interplay between matrix electromechanical properties, cell adhesion, differentiation, and the mechanical properties of cardiomyocytes. First, we will develop and deeply characterize a new cell-matrix platform that mimic the *in vivo* environment; second, we will determine the optimal culture conditions to promote organotypic assembly of seeded cardiomyocytes.

Context and objective:

Recent advances in the field of induced pluripotent stem cell (iPSC) research and establishment of robust cardiac differentiation protocols from iPSC have significantly changed our perspectives on cardiovascular research by providing us the possibility to generate patient-specific cardiac cells to explore disease etiology, develop novel drugs, and cell replacement therapies. Likewise, the remarkable progress in tissue engineering allowing the development of cardiac tissue that comes close to reality participates also in this profound change in the field. Tissue engineering relies upon the promotion of cells growth on a bio-inspired scaffold that mimics the extra-cellular matrix (ECM) of the tissue. **The goal of this project is to develop a new generation of scaffolds for heart tissue** for which specific physical properties are necessary.

Myocardial tissue shows a hierarchical structure with aligned fibrous cells embedded into 3D honeycomb-like micro-patterns formed by collagen fibers (PMID-18978786). Thus, a scaffold with fibrous structure is crucial for cell organization, survival and function of the seeded cells. To respect anisotropic organization of cardiac tissue and promote development of functional syncytium, the fibrous structure needs to be aligned. Furthermore, the scaffold should fit the elastic mechanical properties of the heart. Finally, the biomaterials need to support cell adhesion, differentiation and proliferation. On the other hand, **heart is a mechanically-active organ** that can sense and responds to its environment. Indeed, it relies on a complex 3D network to maintain appropriate function. One key mechanism in the 3D functional hierarchy of the heart is the propagation of mechanical forces through cardiomyocytes. The cardiomyocytes transduce these mechanical forces to chemical and electrical responses. Hence, **controlling the conductivity in the scaffolds is of paramount importance** for cardiac tissue engineering, as it will help to promote the **organotypic assembly and function** of microfabricated tissues. The materials that are **traditionally used for cardiac**

tissue engineering do not combine appropriate mechanical and electrical properties. As a result, **traditional engineered** tissues are unable to keep up with the pace of contractions of the native myocardium.

Therefore, to develop a fully functional heart-like tissue, the recruited **PhD student will** investigate the **electromechanical stimulation of cardiomyocytes *via* attachment to piezoelectric fibrous scaffolds.** Ideally, it will lead to **improved cellular interaction and synchronized contraction of cardiomyocytes**. Piezoelectric polymers are an attractive class of materials due to their intrinsic ability to generate an electrical voltage in response to a mechanical deformation without external power sources (PMID-20371302). Moreover, the changes in surface charge of the scaffold due to piezoelectric properties can affect protein adsorption, cellular adhesion and cellular activity (PMID-26705272). In the field of cardiac tissue engineering, piezoelectric polymers represent novel promising substrates (PMID-20371302).

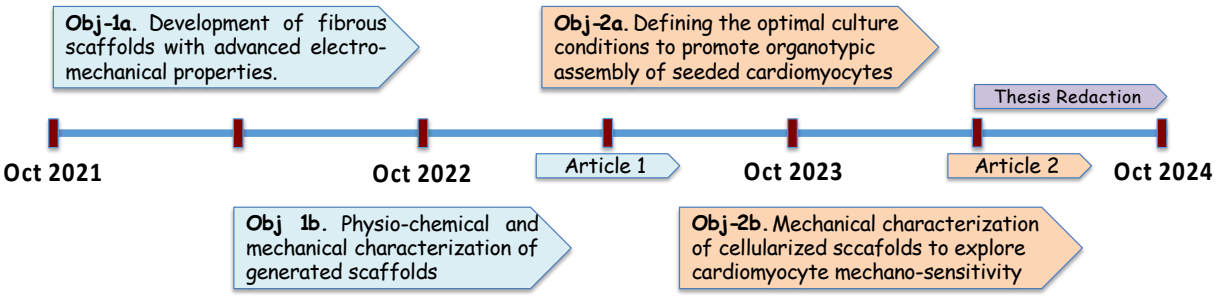
The PhD student will thus develop **scaffolds based on piezoelectric nanofibers** to obtain bio-inspired materials with **improved electro-mechanical properties to explore cardiomyocyte mechano-sensitivity.** The novelty of this project is that we propose to engineer and develop a novel substrate that will combine two essential properties of cardiac tissue: the electromechanical stimulation and the fibrillar structure, both embedded in a unique material. Until now, most research was based on polydimethyl siloxane (PDMS) scaffolds made by soft lithography. However, this technique is limited to working in 2D. To generate the scaffold that mimics the ECM fibrillar structure, **the PhD student will use electrospinning**, a simple and cost-effective technique that can be scaled up for industrial applications (PMID-27826001). Electrospun nanofiber matrices show morphological similarities to natural ECM. In fact, they are characterized by ultrathin continuous fibers, high surface-to-volume ratio and variable pore size distribution.

Specific objectives of this PhD thesis will be;

Objective-1-Development of fibrous scaffolds with advanced electromechanical properties resembling the extracellular matrix. We will use Poly(vinylidene fluoride) (PVDF) which is a semi-crystalline polymer exhibiting electroactive properties. Indeed, PVDF has five distinct crystalline phases: α-to-ε, where β-phase exhibit the higher electroactive properties. Usually the piezoelectricity is introduced by poling a PVDF membrane. By using electrospinning, we will confer piezoelectricity to our fibers *in situ* during their formation *via* controlling the electrospinning parameters. Taking advantage of this, we will **generate an aligned network of fibers to mimic the native myocardium structure**. In parallel, we will functionalize the scaffolds using **plasma treatment** to enhance their physicochemical properties and induce hydrophilicity. Our lab is already equipped with an electrospinning system that can make aligned fibers, and preliminary work has confirmed both the piezoelectric properties of the fiber network generated and the efficacy of plasma treatment for cell adhesion. The fibrous scaffolds will then be characterized by scanning electron microscopy (SEM, topography), and X-ray diffraction (PVDF crystalline structure and dipole orientation). Moreover, **mechanical measurements** (rigidity, viscosity, adhesion) will be performed both in the dry scaffold and in the cell culture medium using **atomic force microscopy** (AFM). AFM is the perfect technique to characterize the mechanical properties with nanometer resolution, and it will be critical to orient the design of performant biosubstrates in this project.

Objective-2-Defining the optimal culture conditions to promote organotypic assembly of seeded cardiomyocytes. To test the propensity of cardiomyocytes to colonize the scaffold developed in Objective-1 and to form a functional heart-like tissue, we will vary several parameters, including porosity, fiber ultrastructure, seeding conditions (e.g. low oxygen concentration) and culture time. In addition, the PhD student will test the impact of coculture, that is, of adding endothelial cells and fibroblasts to the culture of cardiomyocytes, and how they affect their survival, maturation and organotypic organization. The quality of the developed cultures will be tested by **molecular** (gene and protein expression), **cellular** (survival, sarcomere organisation, formation of cell junctions, cell orientation) and **functional** (calcium handling capacities, contractility) analyses. Finally, we will **measure the forces** that the developed ECM mimicking scaffolds apply to cardiomyocytes using AFM. Indenters of different sizes will be used to study the properties of cells at both the nanofiber scale and at the cell microscale. Moreover, we will correlate mechanical measurements to cytoskeleton organization after cell seeding by using immunolabelling and fluorescence observations on the same cells.

Project workflow:



Justification of suitability for *i-Bio*:

i-Bio addresses fundamental questions in biology related to 4 major fields of exploration. This project fits in the third field of the i-Bio project "adaptation, learning, plasticity". This project aims to develop a new tissue model to address fundamental question about heart structure and function, to better understand the remodeling (adaptation) of heart during cardiovascular diseases and ageing. In the fundamental side, the project proposes to explore the complex interplay between matrix electromechanical properties, cell adhesion and differentiation, and the mechanical properties of cardiomyocytes. Our proposal fits well with the objectives of i-Bio and its interdisciplinary character. Our expertise in the study and therapy of cardiac disorders by employing bio-inspired scaffolds embedded with cells (Partner 1) as well as is our expertise in cell mechanics (Partner 2) are well established. Therefore, our stem cell biology and our *in vitro* and *in vivo* skills are fully complementary with the i-Bio research network. As such, the project fits well with the main objectives of the i-Bio in that it combines basic cell biology and physiology, physics of biomaterials, physico-chemical research and tight collaborations between interdisciplinary groups featuring expertise in each of these areas.

Role of each supervisor / skills provided:

The realization of this project requires close collaboration between the CARTHER team at the Sorbonne Université (IBPS-B2A) and the LAMBE team at the University of Evry-Paris Saclay

(UMR CNRS 8587). The PhD thesis will be directed by Dr Zhenlin Li (CARTHER) and Pr Sid Labdi (LAMBE) with participation of Pr Onnik Agbulut (CARTHER) and Dr Guillaume Lamour (LAMBE).

The CARTHER team has the expertise in the cell preparation (cardiomyocytes derived from hiPSC), tissue engineering (electrospinning), cardiovascular physiology and cell-based assay. The LAMBE team has expertise working with the Atomic Force Microscope to study the nanoscale biophysical properties of living cells and also ECM characterization. The collaboration with the LAMBE team will allow performing mechanical measurements in order to assess both the elasticity of the biomaterials and of the cardiac cells.

Profile of the desired student:

This project will be the ideal platform for **a polyvalent, multifaceted candidate with a strong desire to acquire a triple culture** at the interface of biomaterials, cell biology, and cell electromechanics. The ideal candidate will have a biophysics/bioengineering background, with good understanding of both cell biology and cell biophysics. However, a strongly motivated candidate with either a major in biomaterials or physical chemistry could also fit.