HYPERSECRETION IN SEVERE AIRWAY DISEASES: AN INTEGRATED BIOPHYSICAL APPROACH OF THE MUCOCILIARY ASPECT OF THE BRONCHIAL EPITHELIUM

1. DESCRIPTION OF THE PHD THESIS PROJECT

1.1 OBJECTIVES OF THE PROJECT BASED ON THE CURRENT STATE OF THE ART

Severe chronic respiratory diseases affect hundreds of millions of people worldwide. They are associated with an impaired mucociliary clearance, which results in a sharp increase in susceptibility to infections and inflammation. Mucociliary clearance is an essential innate mechanism consisting of the transport, at the surface of bronchial epithelium, of a protective sticky and viscoelastic fluid called mucus. External inhaled pathogens and particles trapped in the mucus are eliminated from the airways via an active transport, which relies on the continuous beating of microscopic cilia located on ciliated epithelial cells. The identification of specific biological pathways involved in the deleterious changes of the mucociliary system allows a better understanding of the abnormal mucociliary clearance. Yet, mucociliary transport relies on physical mechanisms, which involve active cilia-generated forces, hydrodynamic coupling between the ciliary beats and the viscoelastic response of mucus. It is therefore crucial to elucidate these mechanisms.

Epithelium surface. The physical system

The mucociliary system is schematized in Fig. 1. Mucus, secreted at the airway surface is organized in two layers. The top one is made of a shear-thinning gel, which is transported out of the respiratory tract to eliminate inhaled pollutants. The bottom periciliary layer is in contact with the epithelial cells. It consists in a polymer brush, composed of mucins tethered to cilia. Macroscopic transport of mucus is induced by the hydrodynamic force resulting from the collective and coordinated motion of cilia.

Fig. 1 Schematic representation of the two layers mucociliary system

Fluid transport and ciliary activity on reconstituted in vitro human epithelia

Few experimental studies have been dedicated to the physics of the mucociliary system because of the difficulty to perform in-vivo studies. The recent development of primary airway epithelial cells culture at Air Liquid Interface (ALI) by the team of the thesis co-director allows overcoming previous limitations. This in-vitro culture model mimics the mucus-secretory and ciliated surface phenotype expressed in-vivo. It has been reported that the mucus could be circularly transported as a rigid disk over a whole culture chamber. In the team of the thesis director, it was very recently showed that local circular flow patterns also spontaneously arise during ciliogenesis and the size of these patterns scales with the surface density of beating cilia (paper submitted). ALI reconstituted epithelia from severe asthma patients were shown to be unable to generate macroscopic mucus flow, due to an abnormal low ciliary density. Furthermore, a fine regulation of the local
density of beating cilia and of the directional coordination of ciliary beats was discovered within these circular flow domains. Both quantities progressively increased with the distance to the flow domain centre, suggesting that hydrodynamic forces could affect ciliary beat directions and ciliary density. Yet, the coupling between the cilia and the mucus as well as the dynamics and coordination of the cilia remain poorly understood.

**Physical phenomena**
The first objective of the project is to understand the physical mechanisms underlying the mucus transport at a macroscopic scale. The systems is active, nonlinear and coupled system. Indeed, the beat of an individual cilium generates a local flow, in both top and periciliary fluids, which exerts a hydrodynamic force on distant cilia. This force perturbs their beating motion (direction, phase...), which in turn alters the flow. These hydrodynamic interactions among cilia depend on inter-cilia distances, beat patterns and mucus rheological response.

A critical issue is to understand how cilium-cilium hydrodynamic interactions affect their activity and whether they are involved in the coordination of the motion of distant cilia. Mechanosensing is also a candidate for cilia coordination. Indeed, the hydrodynamic force exerted and sensed on each cilium of a given ciliated cell might be transduced into a biochemical signal activating the ciliated cell. Cells could then be able to regulate the metabolism of ciliary activity (changing beat frequency) or induce transdifferentiation of adjacent cells (e.g. to new ciliated cells). The second objective of the project is to quantitatively describe the relation between ciliary activity, mucus rheological properties and mucus transport. The goal is to provide a quantitative framework to interpret abnormal mucociliary transport from the observation of morphological and functional defects of the bronchial epithelium in chronic respiratory diseases.

**Nano health axis: Biological monitoring is challenging in the case of nanoparticles exposure, and a bottleneck is to understand inhaled dose and deposition.** The deposition of nanoparticles in airways is an important mechanism behind chronic airway diseases exacerbations and development, that is the reason why nanoparticle effects and deposition in epithelial cultures obtained from healthy, smokers and subjects with various chronic airway diseases will be studied in the present proposal linking this to nanohealth.

The goal of the proposed project is to investigate the intricate interplay between the collective dynamics of the cilia and the transport of mucus, which results in the formation of patterns at the micro/macroscopic scale. We want to identify the abnormal physical properties characteristic of the mucociliary transport in the context of severe asthma.

### 1.2 METHODOLOGY

**Experimental research**
Here we propose a quantitative experimental study to address the question of the coupling between the mucus transport and the ciliary activity. Our multidisciplinary approach will be based on a large panel of human bronchial ALI cultures of control subjects and patients with severe asthma, pieces of tracheas provided by surgeons, coupled with rheometry, advanced optical microscopy techniques and image processing.

- Bronchial ALI cultures of various geometries obtained from endobronchial biopsies will be prepared in the lab of the co-director P. Chanez. The maturation of the reconstituted epithelium and the emergence of surface mucus flow during ciliogenesis will be monitored.

- Rheological properties of the mucus secreted in the various ALI cultures will be studied in a dedicated rheometer in collaboration with Dr. Favier (Laboratoire M2P2, Marseille).

- The ciliary activity will be assessed in the lab of the thesis director using optical microscopy on living ALI cultures. Confocal microscopy will be performed to image beating and immobile cilia. DIC optical microscopy will be used to study ciliary beat synchronization. Bright field microscopy coupled with image processing will allow measuring ciliary density, beat direction and frequency. Epithelium morphology will be studied with immunohistochemistry and immunofluorescence.

- Surface and periciliary flows will be determined by Particle Image Velocimetry (PIV) performed and processed in the lab of the thesis director in CINaM.
Methodology or process used

The proposed approach is summed up in Fig. 2. We will study surface flow and mucus transport by tuning three parameters acting on hydrodynamic interactions: the ciliary activity, the surface and periciliary rheological properties and the boundary conditions. We will quantify the interplay between the ciliary activity and the spatio-temporal evolution of mucus transport. We will study ciliary activity as a function of the severity of the respiratory disease and of the phenotype of the patients to establish an “ID” card of patients which links the phenotype to ciliary activity and mucus transport. The coupled feedback of hydrodynamics on ciliary activity will be studied during ciliogenesis. We will expose several ALI cultures at an early stage to different hydrodynamic conditions and compare the temporal evolution of their ciliary activity. This will allow us to characterise the role of the mucus and its rheological properties on ciliary activity, thus on mucus transport.

Fig. 2 Schematic of the interplay between active cilia and mucus transport - The coupling in the mucociliary system arises from the hydrodynamic interactions. The tunable parameters are the cillum activity, the boundary conditions of the culture chamber and the rheological properties of the periciliary and surface fluids. The possible role of mechano-transduction mechanisms on the coordination/synchronisation of the cilia will be tested by applying an external hydrodynamic force.

1.3 WORK PLAN

We briefly detail the different tasks

**ALI Culture** – Samples of various diameters (12 or 24mm) and geometries (linear channel or ring) with well-characterized phenotypes will be prepared an used at day 14th (cilia apparition) and studied until day 35th.
These ALI cultures are routinely used in P. Chanez’s lab. The secreted mucus will be periodically collected at the surface of the cultures and its rheological properties determined in a cone-plate rheometer.

**Surface flow patterns and mucus transport** – Size, direction and velocity of both surface and periciliary fluids will be quantified on each culture chamber, and local flow patterns characterised. Experiments will be also done on pieces of epithelium peeled from pieces of trachea provided by surgeons. For that purpose we will use fluorescent nanobeads (that enter into the periciliary layer) and microbeads on the surface fluid and track them. We will determine whether the presence of local circular surface-flow domains reported in circular chambers is an intrinsic hydrodynamic phenomenon or is driven by the geometry of the epithelial culture. The surface fluid rheology will be varied by diluting native mucus, or by replacing it by a Newtonian fluid or a gel (agarose).

**Ciliary activity** – The temporal evolution of the ciliary activity on each flow domain will be followed (from day 14th to day 35th) under different hydrodynamic conditions by varying the rheological properties of the surface fluid or by applying controlled external surface flows at the epithelium surface. The temporal evolution of ciliary density, of phases, directions and frequencies coordination of ciliary beats, and of the flow domain size will be measured. The challenge will be to implement innovative image processing algorithms that we can automatically applied.

### 1.4 Supervisors and Research Groups Description

This project is part of an integrative approach, supported by the French National Agency for Research, which involves physicians, biologists and physicists from Marseille and Montpellier to study the dysfunction of the bronchial epithelium in the context of chronic respiratory diseases and which will end at the end of 2017. The project presented here is based on the discovery during the ANR of an original coupling between ciliary activity and mucociliary transport, suggesting the existence of unexplained mechanotransduction effects (currently being published). These behaviors need to be detailed, quantitatively described, understood and valued in a clinical setting. This is the objective of this project. A first Cofund ‘prestige’ funding has just been obtained to hire a postdoc in 2017 to develop new observation tools and to start a first measurement campaign.

The strong advantage of our project relies on its multidisciplinary and on the availability of human samples with well-characterized clinical phenotypes thanks to the collaboration of the renowned team of physicians led by P. Chanez in the field of chronic respiratory diseases with the group of physicists led by A. Viallat.

The research group of Annie Viallat belongs to the new Departement ‘Physics and micro-nanoEngineering of living systems’ in CiNaM. It is a very active partner, has facilities for cell culture and has excellent equipment for light microscopy and Velocimetry measurements. The group has great expertise in biophysics, soft matter physics, and physics of active matter. The group has recently highlighted novel effects on the coupling between ciliary activity and mucus transport in chronic respiratory diseases.

The research group of Pascal Chanez

2. 3I DIMENSIONS AND OTHER ASPECTS OF THE PROJECT

2.1 INTERDISCIPLINARY DIMENSION

The project draws its originality from the integrated translational approach ‘from bench to bedside’ and is interdisciplinary by essence. The group led by A.Viallat combines the expertise on soft matter and complex fluids, fluid dynamics and biomechanics and will be in charge to supervise biophysics analysis (ciliary activity, mucus transport). The group led by P. Chanez masters a model of in vitro and 3-dimensional reconstruction of the bronchial epithelium as seen in human airways. Moreover, the recruitment of asthmatic subjects in Hospital department is responsible for a large number of bronchial endoscopic specimens making it possible to carry out comparative studies between healthy and pathological cells. P.Chanez will supervise the recruitment of patients and production of in vitro reconstituted epithelium.

2.2 INTERSECTORAL DIMENSION:

Our department, at APHM managed by Dr Laurie Pahus (PharmD) participates in more than 30 clinical trials with industrial or academic sponsors. Our main activity focuses on severe asthma, COPD and idiopathic pulmonary fibrosis. About 100 patients are monitored monthly. Many unmet needs exist for the therapeutic management of these chronic respiratory diseases. In order to identify new targets for future innovative treatments and more powerful diagnosis tools, both fundamental and clinical researches are needed. Thereby, we develop several translational research projects. The success of this project depends on the access to the largest number of human bronchial epithelium samples and sputum samples. These samples are collected in our department on clinical research participants after their appropriate consent. We have the ability to collect adequate biological samples using for example bronchial endoscopy or induced sputum techniques.

2.2 INTERNATIONAL DIMENSION:

The student will participate to several international conferences on the subject. He will participate to the Gordon Research conference on Cilia, Mucus and Mucociliary interactions held every two years and to the annual conference of the American Physical Society, division of Fluid Mechanics. The student will be welcome in various laboratories within Europe including Dr Charles Pilette in Belgium and Dr Jamila Chakir in Quebec. In these laboratories, he will learn some new techniques related to his project on epithelium developed in our Laboratory.

3. RECENT PUBLICATIONS

Ciliary activity:


Epithelium model


Severe asthma (2016)


Gras D, Chanez P. New sociology for better understanding severe eosinophilic asthma: introducing the SOCS family. Eur Respir J. 2016 Sep;48(3):608-10.


### 4. Expected Profile of the Candidate

The candidate will have a solid background in experimental physics and a passion for pluridisciplinarity. He will have a strong interest for active matter, soft matter, biology and medicine. He will have an academic background of physics and/or medicine. He will have experimental skills on optical microscopy. Skills on image analysis, data processing will be appreciated. The knowledge of ImageJ, matlab, or langage of programmation like python will be useful.

The candidate must be sociable, like to discuss and make the interface between different and complementary scientific cultures. On a daily basis, he will have to interact with physicists, biophysicists, biologists and doctors.
Annie Viallat’s profile

Biography:
Annie Viallat is engineer of Ecole Polytechnique (Paris, France). She received her PhD in Physics from the University of Grenoble (France) in 1987, working on polymer gels and NMR. After a postdoc on theory of conjugated polymers in the Materials Departments (UC Santa Barbara), she joined the Spectrométrie Physique lab (Grenoble) in 1989, studying polymer gels and heterogeneous polymer solutions. Her research moved to biological physics in 1999. From 2005 to 2015 she led a group in Marseille on the dynamics in microflows of vesicles and blood cells. Since 2016, she is the head of department ‘Physics and Engineering for Living Matter’ in CINaM (Marseille), working on active matter, microcirculation of red blood cells in disease and physics of the mucociliary clearance.

Positions:
2016 - Head of department at CINaM Active Matter, biological physics, biological fluids,
2007-2014 Director of the CNRS French Consortium ‘Physics from Cell to Tissue’
1999-2002 Group Leader, Laboratoire Spectrométrie Physique, Grenoble, Polymers, Giant Lipid vesicles
1989-1999 Junior Research Scientist, Laboratoire Spectrométrie Physique, Grenoble

Cursus
1982 Ingénieur Ecole Polytechnique, Paris
1984 Ing. Génie rural, Eaux et Forêts, Paris
1984 DEA Physique du solide, Paris
1987 Doctorat de physique, 1987, Université de Grenoble
1994 HDR, Grenoble

Current research area: Active Matter - Biofluid Physics – Cell Rheology – Fluid and elasticity in living systems:
- Soft micro-shells, artificial capsules, liposomes, encapsulation.
- Red blood cells dynamics in flow: Lift force – Cell Biomechanics –Sedimentation – fluid structure interaction
- Blood cell dynamics in the microvasculature: Microfluidics- red blood cells in submicron splenic slits - vasoocclusion in sickle cell anemia, blood cells adhesion and margination
- Physics of the mucociliary transport: collective behaviour of bronchial cilia, flow patterns, coupling between ciliary activity and mucus transport

Relevant thesis supervision history since 2000. Duration of thesis 3 years except for ZH Huang and K Khelloufi (4 years)
2005: UJF J. Dalous , Researcher and now physician, publications: 1 Biophys. J
2008: U. Méditerranée, ZH Huang back to Taiwan, publications : 1 Soft Matter, 1 New J. of Physics
2015 : Univ. Salvador de Bahia L Bonfim , enseignant university Brasil, publications : 1 PlosOne,
2015 : AMU K. Khelloufi Ingénieur Platod (Startup Paris), publications : 1 European Respiratory J.(proceeding), eLife submitted, other publication in preparation

No thesis currently supervised.
Pascal Chanez’s profile

Biography
Dr Pascal Chanez MD, PhD, FERS is a consultant and full Professor of Respiratory Medicine attending the « Clinique des bronches, de l’allergie et du sommeil et de la plateforme ambulatoire du pôle thorax » at the APHM and Aix Marseille University at Marseille France. He coordinates a research group at INSERM-CNRS U1067 in the same institute on the role of bronchial epithelium in inflammation and environmental aggression in severe bronchial diseases. He is the head of a clinical research group investigating new innovative treatments for severe asthma and COPD. He gained his MD and PhD degrees from the University of Montpellier and was a fellow at the Imperial College in London UK with Pr PJ Barnes. He is the authors or co-author of more than 300 peer reviewed articles, reviews and monographs (H-index (Web of Science): 60: see pubmed n=388) He is an editor of the European Respiratory Journal and Journal of allergy and clinical Immunology . His clinical and research interests are devoted to a better understanding of the mechanisms of severe asthma and COPD with a special effort put into bringing clinical and biological findings together to provide to the patients new specific biomarkers and therapies. He has demonstrated in the past his willingness and ability to gather researchers and clinicians together to decipher the complexity of severe asthma in France and Europe.

Cursus
2008-2016: Department of Pulmonology, Head, APHM Aix Marseille University Group Leader INSERM U1067
2015: Professor of medicine "classe exceptionnelle” Aix Marseille University
2010: Professor of medicine "1er "Aix Marseille University
2008: Full Professor of medicine PU "2ème Aix Marseille University
1988-2007: Attending physician PH CHU Montpellier
1983-1988 Medical residency CHU Montpellier
1987: Research fellow Imperial College London UK
1977-1983: Medical Student CHU Montpellier

Diploma
1994 PhD University of Montpellier,
1994 HDR University of Montpellier
1988 Specialist in Pneumology University of Montpellier
1988 MD University of Montpellier

Grants
Grants EU: ENFUMOSA1996 coPI, BIOAIR 2006 coPI, UBIOPRED2009 IMI coPI, AIRPROM 2010 coPI
Many industrial grants from different drugs companies including some unrestricted grants for research

Awards/Lectures
• 1988 : Lauréat de la Faculté de Montpellier I, Prix de thèse Armet
• 1988 : Médaille d’or des Hôpitaux de Montpellier, lauréat des Hôpitaux
• 1989 : International travel grant (AAA); 1990 : Prix Allergie 2000
• 2014 : FERS; 2016; prix de la Fondation de la recherche médicale prix BPCO Pettay

Relevant thesis supervision history. Publications with all students
• Nathalie Carayol (2001), post-doc at Collège de France (Paris)
• Caroline Bonnans (2002), CR2 Inserm (non active status, Rinat Pfizer San Francisco)
• Arnaud Bourdin (2006), PU-PH (CHU Montpellier and Inserm U1046)
• Delphine Gras (2007), post-doc UMR Inserm U1067 (Marseille)
• Yaël Gernez (2011), Resident Physician at Alameda County Hospital (San Francisco)
• Khuder Alagha (2015), MD (Montpellier)