Postdoctoral position on the development of cryogenic electron microscopy and spectroscopy methods for the study of extracellular vesicles at bone tissue interfaces

Workplace: NANTES; **Proportion of work:** Full time
Contract Period: 18 months (extension possible); **Starting date:** 1 April 2021
Salary: between 2675 and 3977 euros gross monthly depending on experience

Objectives:

The main goal of the project is to develop and apply cryogenic electron microscopy and spectroscopy techniques to investigate beam sensitive extracellular vesicles (EVs) involved in the processes of bone remodelling and repair. EVs are produced in bone tissue in response to many stimuli. They are key players in cell-to-cell communication and their functional role depends on their molecular cargo, which includes proteome content. During bone damage, signals for bone repair are triggered. These signals, which have not yet been fully resolved, could involve different type of bone cells and the production of EVs. The osteocytes, which represent more than 95% of all bone cells, are considered as major regulators of bone mass and play a significant role in bone homeostasis and the maintenance of bone integrity. Other type of cells, the osteoblasts, the bone forming cells, synthesize dense cross-linked collagen and proteins which compose the un-mineralized organic portion of the bone matrix (the osteoid) prior to the maturation of bone tissue. Tissue breakdown and micro-cracks target different types of bone cells but particularly target osteocytes. Understanding the role of EVs secreted by bone cells on the processes of bone repair is therefore very important. Questions such as the specific localization of the different specialized vesicles, as observed in-vivo, as well as complete information on their cargo are still unanswered. In this context, high resolution 3D imaging of the ultrastructure of osteoblasts and osteocytes using volume SEM techniques have already been realized, including imaging of the soft and hard bone tissue components at room temperature. 3D reconstructions of the osteocytes network and lacuno-canalicular network with tens of nanometer spatial resolution have already been produced. This project has the ambition to elucidate the precise localisation and chemical content of the different specialized vesicles in bone using a combination of advanced analytical cryo-electron microscopy approaches. Methods will be developed to resolve the ultrastructure of osteocytes with sufficient resolution to identify the EVs.

Job description / Activities:

You will develop novel acquisition and transfer schemes and workflows for cryogenic 3D FIB/SEM data acquisition and analysis and apply them to the identification of specialized vesicles that may be involved in bone remodeling and repair. Tasks include sample preparation using high-pressure freezing /plunging of bone sections and trouble-shooting in cryo-FIB and cryo-FIB lift-out preparation.

You will analyze the chemical content of the different specialized vesicles as well as model systems using ultra-low dose cryo-EDS and cryo direct detection EELS acquisition and analysis methods.

You will have access to unique imaging and analytical electron microscopy capabilities in the IMN. A newly acquired FIB (ZEISS Crossbeam 550L) with EDX and cryogenic capabilities will serve for 3D imaging and spectroscopy of the biomaterials and preparation of a thin cryo-lamella. Further imaging and spectroscopy analysis will be performed using scanning transmission electron microscopy (STEM) and electron energy loss spectroscopy (EELS) in cryogenic conditions. The Nant'Themis (S)TEM (Thermo Fisher Scientific Themis Z G3) will be used, equipped with specialized holders for cryo-EM (-178 ºC), low dose mode and highly sensitive detectors (direct detection of electrons) coupled with the EEL spectrometer.

Fresh bone sections containing the relevant active interfaces will be provided through close collaboration with Pr. Valérie Geoffroy and Dr. Angélique Galvani (INSERM U1229, RMeS - REGOS team). A strong collaboration within the PMN group in IMN is also expected with Pr. Jean Le Bideau (UMR 6502, IMN - PMN group).

This appointment for an initial period of 18 months could be prolonged through a different project.

Profile and requirements:

You hold a PhD in the life sciences, physics, engineering or related disciplines.
You have excellent experience in sample preparation and characterization by scanning electron microscopy and focused ion beam, FIB/SEM, (instrument and dedicated software) as well as quantitative image analysis and TEM sample preparation by FIB lift-out.
You have at least several years of experience in cryogenic methods applied to electron-beam sensitive materials and in 3D FIB-SEM data reconstruction
You are enthusiastic and greatly interested on performing accurate experimental work using advanced equipment, experimental design as well as data processing methods.
You would like an interdisciplinary project with future clinical applications.
You are quality-oriented, conscientious, creative, and cooperative, with a taste for scientific rigor.
You are able to communicate to different audiences and have high level of English.
You have experience interacting with biologists.
Experience in instrumentation optics, cryo-FIB lift-out sample preparation, cryo-EM sample transfer and knowledge of EVs research or cell molecular biology would be a plus without it being mandatory.

Work Context / Scientific framework of the position:

This postdoc is funded as part of an ANR JCJC project (VINCI project, PI: P. Abellan).

The host laboratory is the Institute of Materials Jean Rouxel (IMN, UMR 6502, http://www.cnrs-imn.fr). The IMN is a joined research centre between CNRS and the University of Nantes, composed by over 200 staff members including over 120 permanent staff members and around 80 PhD students and postdocs. The postdoctoral researcher will benefit from interaction with numerous colleagues working on a number of fields of material sciences through experiments using a myriad of advanced characterization techniques and through simulations. Through the VINCI project, the postdoc will be part of an advanced electron microscopy group, aimed at the elucidation of material structure and composition down to the atomic scale, using newly acquired cutting-edge electron microscopy capabilities in the Pays de la Loire. Within IMN, the postdoctoral researcher will join the PMN (Physics of materials and nanostructures) team, as part of a research axis aimed at the study of biomaterials and their interfaces at the nanometer scale.

The successful candidate will collaborate closely with the Regenerative Medicine and Skeleton (RMeS) laboratory, UMR Inserm U1229 (https://rmes.univ-nantes.fr/). The RMeS laboratory is a joint research unit (Inserm, University of Nantes, Oniris) and a center of excellence and national leader in skeletal ageing and regenerative medicine. Its themes range from the study of the mechanisms that govern the development, growth and aging of bone and joint cartilage to the development of innovative 4R (Replacement, Repair, Regeneration, Reprogramming) skeletal treatment strategies. Work at RMeS will be undertaken within the REGOS ("Regenerative medicine of bone tissues") team, focused on the molecular aspects of aging and bone pathophysiology and on the development of hybrid biomaterials for bone regenerative medicine.

Both laboratories are located in the Nantes area, with easy permutation (20 min, single tram line) using public transport.

Geographic location: RMeS: map here ; IMN: map here

Contact: For any additional information on the project and/or recruitment process, please contact Dr. Patricia Abellan (patricia.abellan@univ-nantes.fr). All applications must be sent through the “portail emploi CNRS” and must include a CV and a cover letter outlining your motivation.