

RENEWALS gRaphENE for WAter in Life Sciences

The structure-activity relationship of biological molecules cannot be entirely accessed by dissecting the cellular milieu. The emerging holistic vision of biology should guide methodological and technological advances for cellular analysis, and, more importantly, the complementation and synergic interplay between state-of-the-art microscopy and spectroscopy tools. The need for analyzing biological samples in comparable physiological environments is at the base of the synergism. The goal of RENEWALS is to develop and implement environmental liquid cells, using photon and electron transparent graphene membranes (Graphene Liquid Cells, GLCs), suitable for cross-talk characterization of bio-cellular samples with atomic force, photon and electron-based microscopies at Elettra, Charles University and National Institute of Materials Physics.

FE-SEM for morphological and chemical analysis





Cellular Sample Preparation and Biological Assays (Structural Biology Laboratory)

(Scanning Electron Microscopy Facility)



TEM and HRTEM for morphological and chemical analysis (Electron Microscopy facility)



X ray Microscopy combined with micro LE-XRF and XAS (TwinMic beamline)





In liquid-AFM and Fluorescence Microscopy (NanoInnovation Laboratory)





GLCs for Nanotoxicology

A tight correlation exists between air pollutants and mortality and morbidity of lung diseases, especially related to the inflammatory processes induced by ultrafine NPs. **AI NPs** are potential biofuel additives and therefore air nano-pollutants. The interactions between AI NPs and lung cells are mediated by species adsorbed onto the NPs in the biological environment (biomolecular corona/shell from Lung Lining Fluids), rather than by the pristine surface properties.



WP1. Design and fabrication of GLCs

Possible fabrication scheme of handable GLCs by DXRL



WP2. Characterization of 10-50 nm Al NPs upon interaction with Lung Lining Fluid (LLF) compared to pristine Al NPs



Morphologic characterization of Al NPs (pristine and shelled with LLF): TEM, FESEM, AFM.

Chemical characterization of Al NPs (pristine and shelled with LLF): FTIR spectroscopy, UV-Raman, visible fluorescence microscopy (of Al NPs incubated with labelled SP-B.

Characterization of oxidation state of Al NPs (raw and shelled with LLF): STXM and LEXRF.

WP3. Cellular Internalization of Shell Al NPs: localization and cell adverse effects



Human lung adenocarcinoma A549 cell line incubated with shell Al NPs in simulated LLF A549 cells after Al Nps incubation in GLCs for multi-method characterization

- Morphologic characterization of pulmonary cells upon incubation with Al NPs (both dried and hydrated conditions in GLCs), and identification of the local accumulation of Al NPs: STXM, LE-XRF, TEM, FESEM, AFM, deep-UV Raman microscopy, visible fluorescence microscopy.
- Evaluation of the possible toxic effects on pulmonary cells upon Al NPs exposure: FTIR Microscopy and dye specific cytotoxic assays (MTT and LDH assays).

