Development of innovative pH sensor to evaluate phagocytosis of nanoparticles

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Introduction: Inhaled nanoparticles (NP) exhibit variable toxicity levels which mainly depend on their physicochemical characteristics (size, morphology, crystallinity, chemical surface composition…). Biological effects monitoring thanks to usual tests (ROS, TNFα, LDH) are performed on alveolar macrophages collected from the respiratory system. In this frame, evaluation of NP uptake by macrophages appears as complementary data useful for NP toxicity assessment.
The aim of this work deals with the development of pH sensible NP allowing the quantification of NP phagocytosed by macrophages, specially the step of fusion between phagosomes and lysosomes.

Material and methods: Two types of fluorescent NP with variable and well-characterized sizes and surface coatings have been synthesized. One type with FITC fluorescence and one other with two fluorochromes (FITC within the core and pHrodo in the porous polysiloxane shell). Red fluorescence of pHrodo probe increases as pH acidifies allowing the distinction of intra-lysosomal engulfed NP in macrophages (cell line RAW 264.7). Observations were realized by confocal microscopy performing fluorescence spectral analysis. Confocal acquisitions were realized with the two types of NP including fluorescent labeling of cell nuclei with hoechst as well as actin labeling with phalloidin for cellular limit detection.

Results: Incubation of pHrodo NP in different acellular mediums with decreasing pH values showed the emission peak of red fluorescence in acidic conditions. Moreover confocal microscopic observations led to distinguish entirely engulfed NP (yellow labeling due to colocalisation of NP in acidic vesicles) from those which are just adherent to the cell membrane (FITC green labeling).

Conclusion/Perspectives: This study allows the validation of pHrodo NP model and the confocal observation of NP in contact with macrophages. We already developed cytometric quantification methods for micrometric particles, which can now be applied to NP.

Key words: Fluorescent nanoparticles, phagocytosis pH sensor, confocal microscopy.