Development of innovative pH sensor nanoparticles: a new approach to evaluate phagocytosis

Lara Leclerc[1,2,3], Delphine Boudard[1,4,5,6], Jérémie Pourchez[1,2], Valérie Forest[1,2], Laurence Marmuse[7], Cédric Louis[7], Valérie Bin[1,4,5], Sabine Palle[8], Philippe Grosseau[3], Michèle Cottier[1,4,5,6].

[1]LINA Laboratoire Interdisciplinaire d'étude des Nanoparticules Aérosolisées, F-42023, Saint-Etienne, France

[2]Ecole Nationale Supérieure des Mines de Saint-Etienne, Centre Ingénierie et Santé, F-42023, Saint-Etienne, France

[3]Ecole Nationale Supérieure des Mines de Saint-Etienne, LPMG, F-42023, Saint-Etienne, France

[4]Université de Lyon, F-42023, Saint-Etienne, France

[5]Université de Saint-Etienne, Jean Monnet, Faculté de Médecine, F-42023, Saint-Etienne, France

[6] CHU de Saint-Etienne, Laboratoire d'Histologie, F-42055, Saint-Etienne, France

[7]NANO-H S.A.S., 2 place de l'Europe, Bâtiment A, Parc d'activité GVIO, F-38070, Saint Quentin Fallavier, France

[8]Université de Saint-Etienne, Jean Monnet, Centre de Microscopie Confocale Multiphotonique, Pôle Optique et Vision, F-42023, Saint-Etienne, France

ABSTRACT

Macrophages (MA) from the respiratory tract constitute the first defense line against inhaled nanoparticles (NP) thanks to their phagocytic activity. The toxicity of NP mainly depends on their physicochemical characteristics (size, morphology, chemical surface groups…). However the relationship between the number of phagocytosed NP and their cellular activity is still unclear. In this context the accurate quantification of internalized NP by macrophages could allow a better assessment of NP toxicity and uptake mechanisms. The investigation of a relationship between NP internalization, physicochemical parameters and the degree of toxicity represents a crucial issue.

This work aimed at developing an innovative nanoscaled pH-sensor allowing the quantification of NP phagocytosed by macrophages based on a system of double fluorescence. Two types of NP of variable and well-characterized features have been synthesized:

- FITC-NP (green fluorescence)
- FITC-NP conjugated with a fluorescent pHrodo™ probe which red fluorescence increases as pH acidifies allowing the detection of intra-lysosomal engulfed NP.

After validation in acellular conditions by spectral analysis in confocal microscopy, the proof of concept of the nano pH-sensor was conducted *in vitro* on a macrophage cell line (RAW 264.7). The detection of entirely engulfed pHrodo-NP (green and red labelings) allowed the quantification of phagocytosis.

The biological effects of nano-pH-sensor (cytotoxicity, inflammatory response and oxidative stress) were also investigated. Results showed that incorporation of pHrodo had no major effects on the cytotoxicity of NP especially for the double fluorescent pH-sensor NP of 250 nm, making them an interesting and powerful tool to quantify phagocytosis *in vivo*.

A better understanding of uptake mechanisms is a key issue of nanotoxicology and these data could be applied to improve drug delivery targeting in anticancerous treatment.

KEYWORDS

Fluorescent nanoparticles, uptake, toxicity.

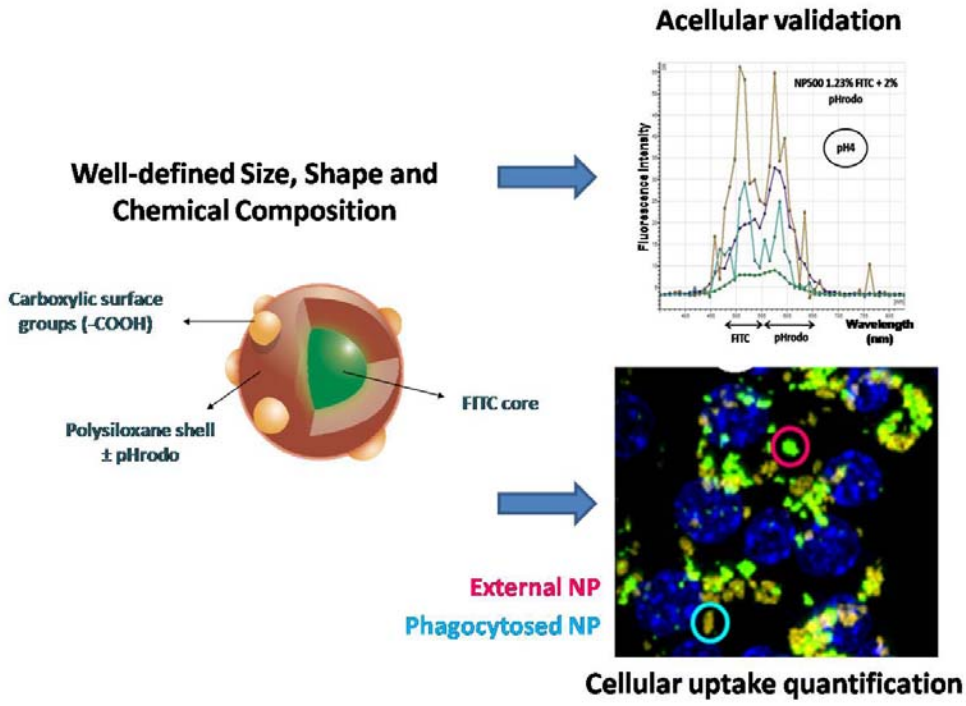

Figure 1 : Nano-pH-sensor.