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

Lara Leclerc, Delphine Boudard, Jérémie Pourchez, Valérie Forest, Laurence Marmuse, Cédric Louis, Valérie Bin, Sabine Palle, Philippe Grosseau, Michèle Cottier

To cite this version:


HAL Id: emse-00583357
https://hal-emse.ccsd.cnrs.fr/emse-00583357
Submitted on 7 Apr 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Development of innovative pH sensor nanoparticles: a new approach to evaluate phagocytosis

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.](image-url)