INTRODUCTION

Application of nanoscale systems such as biodegradable nanoparticles (NPs) to healthcare – referred to as nanomedicine – has the potential to revolutionize the therapy of several pathologies allowing a controlled and targeted delivery of drugs with an improvement of their therapeutic efficiency and a reduction of side effects and toxicity. Among the various routes of administration, delivery to the lung has given rise to a large interest during the past decade for both local treatment of lung diseases and systemic drug delivery. NPs are highly bioavailable after lung administration since there are well-retained in situ and weakly taken up by alveolar macrophages when their diameter is around 250 nm. However, the very same properties that make NPs exciting devices in the field of nanomedicine might induce harmful effects as they interact with specific cells. So far, while toxic effects of inorganic and/or non biodegradable NPs towards the lungs are being progressively documented, little information is available on the effect of biodegradable NPs towards the bronchial epithelium. Because biodegradability does not always mean safety, the toxicity of such nanoparticles should be explored. More precisely, the effect of different physico-chemical properties that are modulated for drug delivery should be investigated. In a previous work, we have highlighted the in vitro safety of poly(lactide-co-glycolide) PLGA NPs on a Calu-3-based model of bronchial epithelium, by demonstrating that NPs did not cause important cytotoxicity or inflammatory cytokines release regardless of their surface properties.

In this work, we have investigated the role of NP surface chemistry and surface charge on the in vitro potential impairment of the bronchial epithelial barrier. For this purpose, three types of surface-modified PLGA NPs were used: positively and negatively charged as well as neutral ones. The Calu-3 cell line cultured at air/liquid interface has been used to mimic the bronchial epithelial barrier. The ability of NPs to diffuse across the mucus layer and their subsequent cellular uptake, as well as their potential effect on the epithelial permeability and the mucus turnover, using MUC5AC as a marker, were assessed. The expression of the corresponding gene was also investigated.

EXPERIMENTAL METHODS

Nanoparticle formulation and characterization

PLGA NPs were prepared by a solvent evaporation technique. Neutral NPs were prepared using polyvinyl alcohol (PVA) as stabilized. To obtain cationic and anionic NPs, chitosan (CS) and Pluronic F68® (PF68) were introduced respectively in the formulation. PLGA covalently linked to rhodamine was used to prepare fluorescent NPs (Rhod-NPs). Size and polydispersity index of NPs were determined using a Malvern Zetasizer Nano ZS and confirmed by TEM microscopy. Surface charge of NPs was determined by measuring their electrophoretic mobility and expressed as their zeta potential.

Cell Culture

The Calu-3 cell line was cultured in DMEM supplemented with 10 % FBS, 50 U.mL⁻¹ penicillin and 50 U.mL⁻¹ streptomycin. To study the epithelial barrier permeability, the cellular uptake and the production of mucus Calu-3 cells were cultured at an air/liquid interface onto polyester Transwell® inserts (0.4 µm pore size).

Transepithelial electrical resistance (TEER)

The formation of a confluent cell monolayer and the influence of NP exposure on the monolayer barrier function were assessed by measuring the TEER using an epithelial voltohmmeter.

Confocal Laser Scanning Microscopy

After 24h exposure to Rhod-PLGA NPs, the cell-covering mucus layer was stained with the alexafluor-488-WGA. Then, the cell layer-supporting membranes of the Transwell® inserts were mounted on microscopes slides. Slides were immediately visualized under a confocal laser scanning microscope.

MUC5AC protein production and gene expression.

Confluent cell monolayers were incubated with PLGA NP. At different time points, the cell layer was washed with PBS to remove the whole mucus layer. Then, cells were lysated. MUC5AC protein was quantified in the washing solutions and in the cell lysates by ELISA test. In another set of experiments, after exposure to PLGA NPs, total RNA was extracted from the Calu-3 cells with TRizol™ reagent and quantitative PCR was performed in a CFX96™ Real-Time thermal cycler (Bio-Rad). Acrolein was used as positive control.

RESULTS AND DISCUSSION

PLGA NPs with different surface properties were designed and characterized. PLGA/PF68 NPs exhibit a
mean diameter of 100 nm as compared to around 200 nm for both PLGA/PVA and PLGA/CS NPs. Zeta potential measurements confirm that stabilizers influence NP surface charge: PLGA/CS NPs exhibit a positive zeta-potential ($z = +32\text{ mV}$), whereas PLGA/PVA NPs are almost neutral ($z = -5\text{ mV}$) and PLGA/PF68 NPs exhibit a negative zeta-potential ($z = -24\text{ mV}$).

Exposure to PLGA/PVA and PLGA PF68 NPs at concentration of 0.2 mg.mL$^{-1}$, which ensured at least the 80% of cell viability, did not exert adverse effects on the epithelial barrier integrity. No significant differences were observed compared to untreated cells. A different behavior was observed after incubation with PLGA/CS NPs which caused a decrease of the TEER associated with an increase of the epithelial permeability. Nevertheless, this effect was transient and reversible (Fig. 1a, b).

Surface properties affected NP interaction with the mucus layer which covers the surface of the cell monolayers and consequently NP internalization. PLGA/CS and PLGA/PVA NPs were partially entrapped in the network formed by the mucus chains while PLGA/PF68 NPs could diffuse unimpeded and were practically absent from the mucus layer after 24h (Figure 2).

Exposure to NPs did not cause any variation either of the MUC5AC intracellular content or of the MUC5AC mRNA level compared to the untreated cells. By contrast, an important increase of both MUC5AC protein amount and gene expression was observed following incubation with acrolein. (Figure 3 and 4)

**CONCLUSION**

Positively and negatively charged as well as neutral NPs penetrate the mucus layer although with significant differences due to their surface properties. Only PLGA/CS NPs influenced the TEER but the effect was transient and reversible. None of the NPs formulations increased MUC5AC mRNA expression or the protein levels. Taken together, these in vitro results highlight the safety of biodegradable PLGA NPs toward the bronchial epithelium and provide a clear evidence of the role of surface coating to design NPs for a controlled local or systemic delivery of drugs.

**REFERENCES**

3. Mura et al., Biomacromolecules 2011

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