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Disintegration of nano-embedded microparticles after deposition on mucus: A mechanistic study

Christian A. Ruge[a,1], Adam Bohr[a,1], Moritz Beck-Broichsitter[a,1], Valérie Nicolas[b], Nicolas Tsapis[a,*], Elias Fattal[a]

[a] Institut Galien Paris-Sud, CNRS, Univ. Paris-Sud, Université Paris-Saclay, 92296 Châtenay-Malabry, France
[b] Microscopy facility, UMS IPSIT (Institut Paris Saclay d'Innovation Thérapeutique), Univ. Paris-Sud, Université Paris-Saclay, 92296 Châtenay-Malabry, France

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ABSTRACT

The conversion of colloidal drug carriers/polymeric nanoparticles into dry microparticulate powders (e.g., by spray-drying) is a prominent approach to overcome the aerodynamic limitations of these formulations for delivery via inhalation. However, to what extent such nano-embedded microparticles disintegrate into individual/intact nanoparticles after contacting relevant physiological media has so far not been addressed. Polymeric nanoparticles were spray-dried into nano-embedded microparticles (NEMs) using different amounts of trehalose as embedding matrix excipient. Formulations were characterized and then evaluated for their disintegration behavior after aerosolization onto model mucus. Although a rapid and complete aqueous redispersion was observed for specific excipient/nanoparticle weight ratios (i.e., greater than 1/1), the same formulations revealed no disintegration after deposition onto a static mucus layer. Double-labeled NEMs powders (i.e., dual color staining of polymeric nanoparticles and trehalose) demonstrated rapid matrix dissolution, while the nanoparticle aggregates persisted. When deposited onto agitated mucus, however, sufficient disintegration of NEMs into individual polymeric nanoparticles was observed. These findings indicate that mechanical forces are necessary to overcome the attraction between individual nanoparticles found within the NEMs. Thus, it remains questionable whether the lung mechanics (e.g., breathing, mucociliary clearance) acting on these formulations will contribute to the overall disintegration process.

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1. Introduction

Polymeric nanoparticles (NPs) are of significant interest for inhalation pulmonary drug delivery applications [1,2], due to their versatility to encapsulate water-soluble or poorly water-soluble drugs [3], and their ability to overcome biological barriers within the lungs (e.g., mucus) [4]. They further facilitate accumulation in alveolar macrophages or prevent clearance, depending on their size and surface properties [5,6]. Thereby, delivery of the encapsulated drug to the desired site of action (e.g., macrophages, interstitium etc.) can be achieved.

The unique physical properties of common colloidal formulations preclude lung deposition, as sizes of around 100–200 nm are expected to be mostly exhaled following inhalation [7,8]. Nebulization of aqueous NP suspensions [9] and aerosolization of nano-embedded microparticles (NEMs), also called Trojan particles [3,10], represent suitable delivery mechanisms to generate adequate particle sizes to achieve deposition of polymeric NPs within the respiratory region of the lungs. Due to the limited physical and chemical stability of aqueous NP suspensions during storage (e.g., hydrolytic polymer degradation and drug leakage) [11,12] and the potential aggregation and concentration of the colloidal formulations during the nebulization process [13], dry powder aerosolization of NEMs is generally preferred to deliver polymeric NPs to the lungs. In this respect, spray-drying offers the possibility to convert polymeric NP suspensions into respirable microparti-

Abbreviations: D_{50}, median geometric particle diameter (based on the volume distribution); D_{ae}, median aerodynamic particle diameter; DLS, dynamic light scattering; n.d., not determined; NEMs, Nano-embedded microparticles; NPs, nanoparticles; *p*, probability value; PDI, polydispersity index; SD, standard deviation; SEM, scanning electron microscopy; STED, stimulated emission depletion; TOF, time of flight; wt.%, weight percent.

* Corresponding author at: Institut Galien Paris-Sud, CNRS, Univ. Paris-Sud, Université Paris-Saclay 5 rue JB Cleíment, F-92296, Châtenay-Malabry, France. Fax: +33 1 46 83 59 46.
E-mail address: nicolas.tsapis@u-psud.fr (N. Tsapis).
[1] These authors contributed equally to this work.

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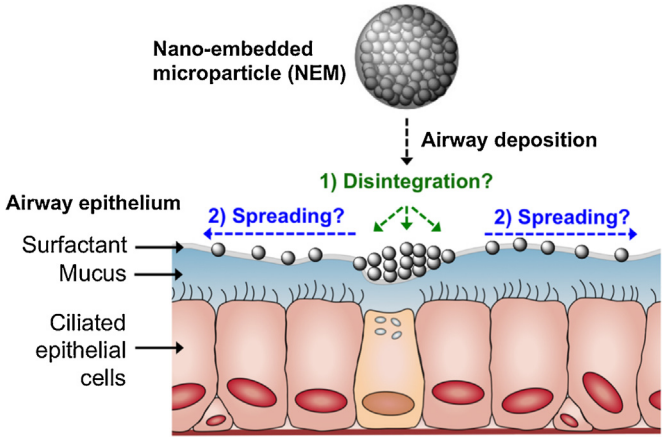

Fig. 1. Post-deposition fate of NEMs on the airway surfaces. (1) After deposition and contact with the respiratory surface (i.e., respiratory mucus), NEMs may release intact polymeric NPs by dissolution of the matrix excipient. (2) Furthermore, the forces resulting from the movement of the lining layer (e.g., breathing cycle and mucociliary clearance) may support the disintegration process and spread the released polymeric NPs along the respiratory epithelium.

cles of sufficient stability [10,14]. Ideally, when deposited NEMs should rapidly release intact polymeric NPs upon contact with the respiratory surface (e.g., mucus) and spread efficiently along the respiratory surfaces in order to cover a large surface area within the target tissue (Fig. 1).

Recent work has identified suitable spray-drying parameters (e.g., inlet temperature) [15,16], excipients (e.g., sugars and polymers) [17,18] and excipient/polymeric NP weight ratios [18,19] enabling sufficient aqueous redispersibility of NEM powders. Surprisingly, investigations on the NEM disintegration process following dry powder aerosolization onto a physiologically relevant medium have so far not been carried out.

To address this issue, a series of NEM formulations composed of standardized polymeric NPs and trehalose as a matrix excipient were prepared by spray-drying in the present study. The resulting powders were characterized for geometric and aerodynamic size, morphology and aqueous redispersibility by means of laser diffraction, time-of-flight (TOF) analysis, scanning electron microscopy (SEM), stimulated emission depletion microscopy (STED) and dynamic light scattering (DLS). Next, defined NEM formulations were deposited onto a model mucus medium (static conditions and under agitation) and their fate was followed by fluorescence microscopy. Finally, double-labeled NEMs were used to account for the observed disintegration process.

2. Materials and methods

2.1. Materials

Fluorescently-labeled polystyrene NPs (PS-NPs; Fluoresbrite® YG Carboxylate Microspheres, nominal diameter of 0.2 μm; λ_{ex} = 441 nm, λ_{em} = 486 nm) in aqueous suspension were obtained from Polysciences (Germany). D-(+)-Trehalose dihydrate (≥99%), sulforhodamine B (75 %) and porcine gastric mucus (type III) were purchased from Sigma–Aldrich (Germany). All other chemicals and solvents were of analytical grade.

2.2. Size and ζ-potential measurements

The mean particle size (i.e., hydrodynamic diameter) and size distribution (i.e., polydispersity index (PDI)) of PS-NPs were measured by DLS (non-invasive back scatter technology (λ = 633 nm), scattering angle of 173°)[20], and their ζ-potential was determined by laser Doppler velocimetry (Zetasizer NanoZS/ZEN3600, Malvern Instruments, UK). All measurements were performed at a temperature of 25.0 ± 0.1 °C using a final sample concentration of ~1 mg/mL (in 1 mM sodium chloride) with at least three runs (duration of 60 s each).

2.3. Preparation of NEMs by spray-drying

Spray-drying was performed on a B-290 (Büchi, Switzerland) equipped with a two-fluid nozzle (inner diameter of 0.7 mm), which operates in a co-current mode using compressed and room air as the atomizing (gas flow rate of ~600 L/h) and drying gas (aspirator setting of 100 %), respectively. Aqueous suspension of NPs was spray-dried at a concentration of 0.2 wt.% with varying amounts of added trehalose (final concentration of 0 (NEM00), 0.1 (NEM01), 0.2 (NEM02) and 0.4 (NEM04) wt.%) at a feed rate of ~5 ml/min. The inlet temperature was set to 85–90 °C, which resulted in an outlet temperature of 40–42 °C. NEM products were harvested from the cyclone and collection vial and then stored under vacuum. Double-labeled NEMs were prepared as described before by supplementing the aqueous feedstock with 0.01 wt.% sulforhodamine B.

2.4. Laser diffraction

The median geometric diameter (D_{50}; based on the volume distribution) was measured by laser light scattering using a Mastersizer 2000 equipped with a Scirocco 2000 dry disperser unit (Malvern Instruments, UK) operated at a pressure of 2 bars. Approximately 10–20 mg of powder was used for the measurements. The obtained diffraction patterns were analyzed in Mie mode with the particle refractive index set to 1.59.

2.5. Aerodynamic properties

The median aerodynamic particle size (D_{ae}) of the spray-dried NEMs was determined by TOF using an Aerodynamic Particle Sizer® (model 3321) equipped with a small-scale powder disperser unit (model 3433) (TSI, USA). For each measurement a few mg of sample were placed inside the disperser, which introduced the NEM powder into the analysis chamber. Analysis run time was set to 20 s.

2.6. SEM analysis

NEM morphology was assessed on a XL FEG 30 Scanning Microscope (Philips, Netherlands). Therefore, NEM powders were spread onto stubs covered with double adhesive carbon tape and then sputter coated with a gold layer using an E5200 Auto Sputter Coater (Biorad, UK). The SEM was operated at an accelerating voltage of 2 kV.

2.7. STED microscopy

The inner structure of NEMs was imaged with an inverted TCS SP8 gated-STED super-resolution microscope (Leica, Germany) using a HCX PL APO 100×/1.40 STED oil immersion objective lens. The instrument was equipped with a WLL l median aerodynamic particle diameter laser (λ_{ex}: 485 nm) and a 592 nm depletion laser. The 592 nm depletion laser was operated at an average power of 200 mW with a gated detection of T_g = 1.5–6 ns.

Trehalose-containing NEMs were dispersed in *n*-hexane on a cover slip, followed by addition of a droplet of immersion oil after evaporation of the solvent. NEMs solely composed of PS-NPs were dispersed in distilled water, followed by addition of a droplet of immersion oil after drying. Samples were imaged immediately after preparation. The sample acquisition was done with the Leica

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