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Generation and delivery of nanoaerosols from biological and biologically active substances

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ABSTRACT

A device for the generation of nanoaerosols from biological substances was designed and the resulting nanoaerosols were characterized. The function of the generator is based on the electrospray-neutralization process in which nanoclusters, obtained by electrohydrodynamic atomization, are neutralized by counter-ions generated by the electrospraying of ethanol at the opposite potential. The device is capable of generating nanoaerosol from suspensions of nanoparticles and solutions of non-volatile organic substances (e.g., antibiotics), proteins, and other biomolecules. This device produces nanoaerosols (10–300 nm in size) with a concentration up to 2×10^7 cm^{-3}. A quartz crystal microbalance based device was designed to monitor the performance of the generator. Using magnetic Fe_3O_4 nanoparticles as reporters, it has been demonstrated that approximately 0.4 μg of nanoaerosol was deposited into a mouse lung in 1 h in a nose-only exposure system. Thus, we present an approach to both generate nanoaerosol from biological and therapeutic substances and to estimate the doses delivered to mice exposed to these nanoaersolized substances.

1. Introduction

Recently, the technology of nanoaerosols has been developed, and drug delivery by this route significantly reduced the required dose of inhaled drugs by several orders of magnitude compared to delivery by the oral route (Onischuk et al., 2008, 2009). The objective of this research is to develop a novel device to generate and characterize nanoaerosols (particles with

Abbreviations: BSA, bovine serum albumin; CPC, condensation particle counter; DMA, differential mobility analyzer; ES, electrospray; ESN, electrospray-neutralization; EtOH, ethanol; HV, high voltage; PMMA, poly(methyl methacrylate); QCM, quartz crystal microbalance; SMPS, scanning mobility particle sizer; WSNF, water soluble nano filter

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size between 1 nm and 100 nm according to the definition given by ISO (International Standardization Organization, 2008)) of antibacterial drugs to potentially treat bacterial infection of the lung.

Using this technology takes advantage of all the benefits of inhalational therapeutics (reduced systemic exposure, highly local concentration of drug delivery to the lungs) plus the benefits of nanoaerosols. Nanoaerosols as a potential route of drug delivery have recently attracted much attention for the following reasons. First, it has been demonstrated that non-steroidal anti-inflammatory drugs delivered directly into the lungs as nanoaerosol particles are effective at a million-fold lower mass dose than with oral administration (Onischuk et al., 2008, 2009). In the latter route, a significant amount of the drug is lost in the liver through the well-documented phenomenon of first-pass drug metabolism (Rowland, 1972). Furthermore, unlike micron-sized aerosols that are predominantly deposited in the trachea and bronchi, nanoaerosols between 10 nm and 100 nm penetrate deep into the lungs and settles in the alveoli (Oberdörster, 2001; Oberdörster et al., 2005). This is advantageous because they can quickly reach the bloodstream and avoid mucociliary clearance. Finally, for the treatment of lung diseases and bacterial infections of the lungs, it is advantageous to deliver the drug directly to the tissue where it is needed.

Despite such obvious advantages, progress in biomedical applications of nanoaerosolized drugs has been slow due to numerous technological problems involving nanoaerosol generation and dosimetry. Most methods used in the generation of nanoaerosol from inorganic materials (such as the evaporation–condensation technique (Mansour et al., 2009)) are not applicable to many therapeutic molecules due to their fragility during the evaporation and sublimation process. It has been shown that even relatively mild ultrasound nebulizers are capable of damaging fragile biomolecules and drugs (Yeo et al., 2010).

Of the different methods summarized in the recent reviews (Biskos et al., 2008; Yurteri et al., 2010) electrospray atomization provides the most efficient way to turn biological or therapeutic substances into nanoaerosol. The first inhaler that employed electrospray atomization was patented by Noakes et al. (1989). Since then, several other designs for nanoaerosol generators have been described – each with an operating principle based on Noakes' electrospray atomization followed by charge neutralization by counter-ions generated in ionized air via radioactive isotopes or corona discharge (Tang & Gomez, 1994; Ijesebaert et al., 2001; Xie et al., 2006; Fu et al., 2011, 2012).

In both of these neutralization techniques, aerosol particles are exposed to highly reactive radicals, hot molecules, ozone, and oxygen atoms, all of which are destructive to biological molecules. A new technique has been suggested for the mild generation of nanoaerosol in which electrosprayed, charged drug nano-clusters are neutralized in gas-phase by counter-ions generated via electrospraying of a volatile solvent, such as ethanol (Morozov, 2011). It was demonstrated that this electrospray-neutralization (ESN) technique allowed for the formation of nanoaerosol containing protein enzymes with significantly higher catalytic activity than in the case of using corona discharge as a source of counter-ions.

However, the aforementioned apparatus (Morozov, 2011) suffered from a few technical challenges: (i) the voltage applied to the capillaries often needed adjustments to stabilize the electrospray during long exposures, (ii) the apparatus sporadically failed to produce nanoaerosol due to unstable electrospray at one capillary, and (iii) the accumulation of deposits on the plastic walls of the electrospray chamber resulted in deterioration of generator efficiency over time. Here, a refined design of the nanoaerosol generator is described which resolves these issues and enables stable aerosol generation for an extended period of time.

Another major challenge with nanoaerosolized drugs arises in measuring and controlling the amount of inhaled doses, i.e., the total amount of nanoaerosolized material deposited in the lungs. Unlike intravenous drug treatments – where all injected drug is delivered to the bloodstream – the inhalation of a nanoaerosolized drug does not always result in systemic circulation; it has been shown that 30–70% of the inhaled nanoaerosol may be exhaled, depending on the particle size (Oberdörster, 2001). Therefore, special devices and techniques are required to estimate the delivered dose. Yet another challenge is the relatively low rate of administration due to the rapid coagulation of nanoaerosol at high concentrations. Thus, at concentrations exceeding 2×10^8 cm^{-3}, more than half of the particles will be lost to aggregation within 10 s (i.e., 1 L of air can only contain $\sim$0.1 μg of particles with a size of 10 nm and 100 μg of particles with a size of 100 nm) (Green & Lane, 1964). Assuming that a human adult inhales 8 L/min, it can be estimated that in 1 h no more than $\sim$50 μg of a drug can be delivered in the form of 10 nm particles or 50 mg in the form of 100 nm particles. For a mouse that inhales only 20 mL/min of air, similar estimates of the maximum dosage results in 0.12 μg for 10 nm particles and 120 μg for 100 nm particles. Considering that 30–70% of inhaled nanoaerosol is exhaled, the maximum hourly doses require appropriate adjustments. Here we report a device with a new approach to generate nanoaerosol from biological and therapeutic substances and a new method to estimate the doses obtained by mice exposed to these nanoaerosolized substances.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), glucose, kanamycin, streptomycin, tetracycline, polyvinylpyrrolidone (PVP, M_w=360,000), dimethyldichlorosilane, iron (III) chloride hexahydrate ($FeCl_3\cdot6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2\cdot4H_2O$) and ethanol were obtained from Sigma-Aldrich (St. Louis, MO, USA). Polystyrene latex particles were obtained from Polysciences, Inc. (Warrington, PA, USA). All chemical reagents were of analytical grade and were used without further purification.