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Generation and Collection of Biological Nanoaerosols

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The report describes new technologies in generation and collection of biological nanoaerosol (NA) developed in the laboratory of Nanostructures and Nanotechnologies, ITEB RAS. NA generator to be used for pulmonary delivery of antibiotics, anti.inflammatory and other drugs and vaccines employs a new atomization principle based on a gas.phase neutralization of electrospray.generated drug nanoclusters with small counter.ions produced by electrospraying of a volatile solvent like ethanol. It was demonstrated that a variety of biological substances including proteins can be atomized into NA of 20-100 nm in size, using this generator without affecting functional properties of the atomized substance. Several approaches have been developed to measure doses of inhaled NA including standard condensation particle counter, quartz resonator and filters from polyvinylpirrolidone nanofibers. It was demonstrated that water-soluble electrospun nanofilters may also be employed to capture pathogens and pathogen biomarkers for analysis of nosocomial infections in clinics wards, in collection of non-volatile biomarkers in the exhaled breath, in testing working places for NA pollution and in other applications.

Keywords: Biological nanoaerosol, Inhaled drugs, Generator, Dosimeter, Electrospun nanofilters, Aerosol collection.

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1. INTRODUCTION

Nanoaerosols attracted a great attention recently for two main reasons. First, products and materials containing nano-components (nanoparticles and nanofibers) aggressively enter the market. These components may eventually become airborne and penetrate into lung. Biological effects of such nanoaerosols (NA), are far from being clear, especially upon a prolonged exposure. Second, NA may be efficiently employed to deliver drugs via pulmonary route. Their ability to penetrate deep into alveoli is beneficial in treatment of lung diseases and allows bypassing destruction in the digestive tract and in the liver upon the first passage. Though advantages of the nanoaerosolized drugs are well recognized [1, 2] their practical usage has been restricted due to lack of good means to produce NA and to determine the inhaled doses.

In this paper we describe our new approaches to generation, detection and dosimetry of biological NA, aiming at development of a new administration route for drugs and other biomedical applications of NA.

2. NANOAERSOL GENERATION

2.1 Principle of Nanoaerosol Generation

A new principle of generation of biological NA is illustrated in Fig. 1. A solution is electrosprayed in a closed chamber and the electrosprayed cloud is neutralized with a cloud of counter-ions produced by electrospraying of a volatile solvent like ethanol. It has been demonstrated that even fragile biomolecules, like proteins retained their structure and functional properties, e.g., enzyme activity in NA produced with this technique [3, 4].

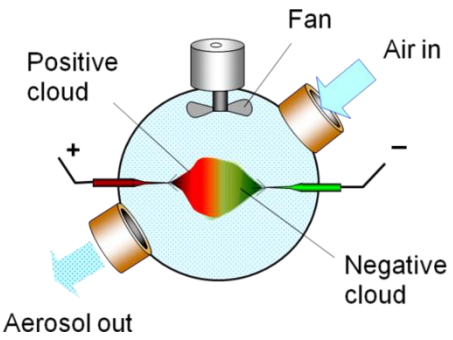

Fig. 1 – Principle of nanoaersol generation by neutralization of a cloud of electrosprayed charged drug nanoclusters from a capillary at a positive potential with a cloud of small negatively charged ions generated by electrospraying of a volatile solvent at a negative potential

Fig. 2 illustrated the last version of a nanoaerosol generator designed in the laboratory for experimenting with small animals. It differs from the earlier versions described in ref. [3] in several improvements aiming at obtaining higher stability, better control of the NA yield and other important functional features. Using solutions of proteins, antibiotics and other substances it was demonstrated that the generator produces NA with a concentration up to 10^7 NP/cm^3 with an average size changing between 20 nm and 100 nm depending on the solution concentration, voltage, airflow and on other

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parameters. Great attention has been paid to develop methods for monitoring NP concentration and doses obtained by animals. In particular, a crystal quartz dosimeter and filter-based probes were developed to measure the overall dose and NA concentrations.

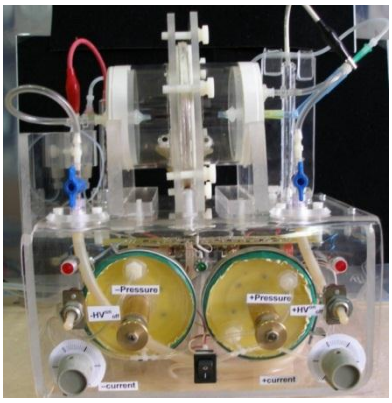

Fig. 2 – The latest prototype of a generator for production of biological nanoaerosol. Air pumps and flow meters are not shown in the picture

2.2 Applications of Nanoaerosolized Drugs

We envisage many potential applications of the nanoaerosol technology both in biomedical research and in medical practice. It has been demonstrated by a group of Russian scientists that when inhaled by mice NA of anti-inflammatory drugs, ibuprofen and indomethacin, were active at doses which are million times lower than those administered orally [1, 2]. This decrease in the doses is partly explained by the fact that the drugs inhaled in the form of NA avoid digestion in the stomach and upon the first passage through the liver. Therefore, the pulmonary route opens an opportunity for other drugs to considerably reduce doses and, hence, the side effects. In collaboration with the National Center for Biodefense and Infectious Diseases of the George Mason University (Manassas, VA, USA) our laboratory explores applicability of the NA generator described above to vaccination and treatment of mice infected with tularemia.

3. COLLECTION OF BIOLOGICAL NANOAEROSOLS WITH ANALYTICAL WATER-SOLUBLE NANOFILTERS

Nanoaerosols cannot be collected using conventional inertial devices like impactors and cyclones. We developed nanofilters from water-soluble polymers to collect micro- and nano-aerosol particles and to transfer them into a small volume of buffer for further biochemical or genetic analysis. Nanofilters are manufactured using a modified electrospinning technology [5-8] in which charged nanofibers are neutralized by a cloud of counter-ions in a way similar to that illustrated in Fig. 1. Such filters enable collection of aerosols at a high velocity (up to 1 m^3/min) and allow to completely transfer the collected material into 10-20 μL of buffer solution upon the filter dissolution. One simple prototype of such nanofilter is presented in Fig. 3. As illustrated in Fig. 4. water-soluble nanofilter collects all micro- and sub-micro aerosol particles with a size exceeding 150-170 nm and most of the smaller NA. Such filters were shown to work at a relative humidity lower than 75 % [5].

Fig. 3 – A simple collector of aerosol and NA based on use of a water-soluble nanofilter

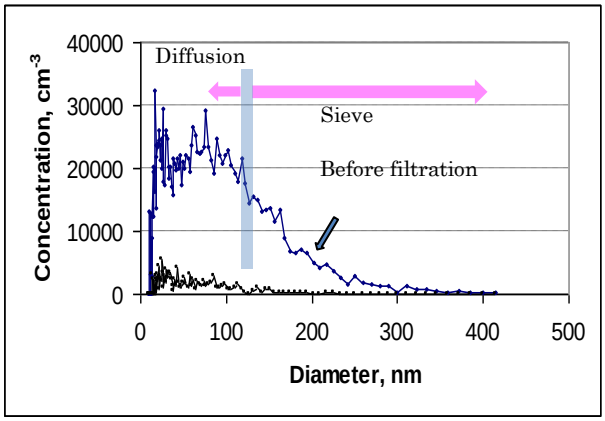

Fig. 4 – Efficiency of NA collection with a water-soluble nanofilter from PVP. Blue line presents NA spectrum generated by the NA generator from a sucrose solution. Black line – spectrum of the same NA after passing through the filter. "Diffusion" and "Sieve" marks denote different mechanisms of NA capturing

3.1 Where Analytical Nanofilters Could Be Used?

These nanofilters are widely used in our laboratory for many purposes: (i) to measure the overall production of NA in the generator described above; (ii) in collection of pathogens and their biological markers indoors; (iii) in collection of "human dust" – micro- and nanoparticles exhaled and lost from skin and clothes. Such filters could also be used for evaluation of air pollution with nanoparticles in industrial buildings [9].

3.2 Collection of Exhaled Non-volatile Biological Markers

One application of water-soluble nanofilters which is under development in our laboratory is a non-invasive technique for obtaining probes of lung liquid by collection of dry residues of lung nanodroplets in the exhaled air. Conventional procedure which is employed for obtaining probes of lung liquid (bronchoalveolar lavage) includes washing lung with a physiological solution. In our approach nanodroplets of lung liquid are first dried by heating the exhaled air and then collected on a water-soluble nanofilter, as illustrated schematically in Fig. 5. Several prototypes of the device have

been tested in the laboratory (see one version illustrated in Fig. 6). As compared to the conventional technique of collecting exhaled breath condensate (EBC) dissolution of water-soluble nanofilter after collection provides much more concentrated samples which require less expensive and sophisticated techniques for analysis of biomarkers.

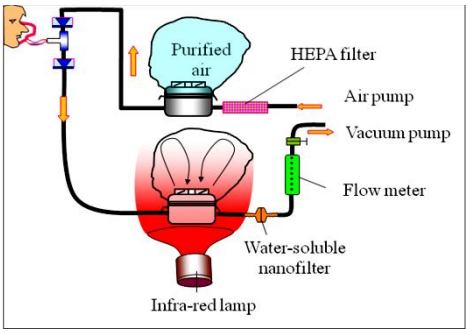

Fig. 5 – Schematic of a device used for collection of non-volatile biomarkers in the exhaled breath using water-soluble nanofilters

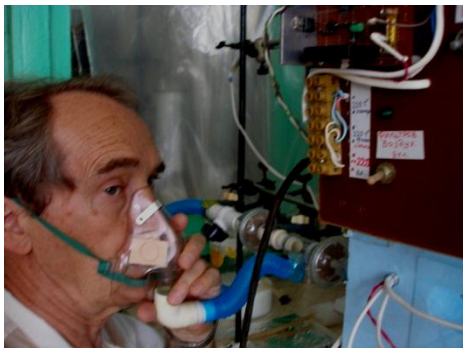

Fig. 6 – Collection of exhaled biomarkers with a water-soluble nanofilter from PVP

3.3 Use of Analytical Nanofilters in Detection of Airborne Tuberculosis Markers

Water-soluble nanofilters manufactured by electrospinning of PVP solutions were also employed to collect aerosol in a tuberculosis clinic. A filter attached to a headpiece of a household vacuum cleaner allowed to collect aerosol at a flow rate of 0.6 m^3/min from a ward volume of 9 m^3 in 15 minutes. Dissolution of the filter liberated all the collected aerosol particles in 0.1-0. mL of water. Quantitative assay of *M. tuberculosis* DNA was based on the real-time PCR using the multi-copy insertion sequence, IS6110, and the single-copy fragment of the regX3 gene as primers and fluorogenic hybridization probes. Effect of the filter material on the assay efficiency was estimated by analysis of a vaccine strain, BCG. Probes were collected in each of the 21 hospital wards and tested for the MTB DNA. In 5 of them occupied by patients (excreting MTB according to microscopic tests) MTB DNA was detected on the nanofilters in the PCR assay. In 4 of these wards ~ 100 copies of the multi-copy primer, IS6110, was discovered. In one ward occupied with a patient having a massive excretion of TB we found as many as $5 \cdot 10^2$ fragments of IS6110 gene and ~ 10 copies of the regX3 gene on the filter. Thus, water-soluble filters enable a simple, rapid and highly efficient technique for collection of aerosols into a small volume of liquid probe. The presence of DNA in the probe could be detected by the real-time PCR assay. Combination of these two techniques allows to control contamination of air with TB and other bacteria and prevent hospital-acquired nosocomial infections.

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