Measurement of Fiber Deposition in a Human Lung Model by Phase Contrast Microscopy with Automated Image Analysis

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Abstract Deposition of fibers in human lungs is known as a health hazard. In-vitro measurements were performed with monodisperse glass fibers in a realistic model of human lungs up to seventh generation of branching to estimate the effect of fiber size and breathing pattern on fiber deposition. Deposited fibers were rinsed from the model segments and gathered on nitrocellulose filters. Phase-contrast microscopy with high resolution camera was used to capture images of filters with fibers. New software was developed for an automated image analysis and local deposition characteristics were calculated afterwards. The whole method proved to be a useful and valuable tool for the evaluation of fiber and particle deposition.

1 Introduction

Fiber is an elongated particle, usually defined by the ratio of the length to the diameter over 3 [6]. Inhalation of fibers is known as notorious health hazard. The research was conducted especially on asbestos fibers, but also man-made vitreous fibers (MMVFs), often used as a substitute for asbestos, has raised the concerns of the potential health hazards [1].

Few experimental works have been performed to characterize the deposition pattern of fibers. Su and Cheng [3] used carbon fibers and performed experiments with constant inspiratory flowrates between 15 and 60 l/min on the human airway replica consisting of oral cavity and following airways up to the fourth bifurcation. Their results proved that most high-inertia fibers were deposited in the oropharynx and the carina ridges of bifurcations. Following comparison [4] with small momentum fibers (TiO_2 and glass) showed that only very few small momentum fibers were deposited in the airway cast. It means they can easily penetrate the upper airway and have a pathogenic effect in the lower airways. Numerical simulation of Zhang et al. [7] implies that lower deposition efficiency of fibers in comparison with spherical particles is probably caused by tendency of fibers to align with the air flow.

2 Methods

A realistic segmented model up to eighth bifurcation was used [2] for measurement with glass fibers (see **Fig 1**). The model is segmented and thus allows easy evaluation of local deposition characteristics.

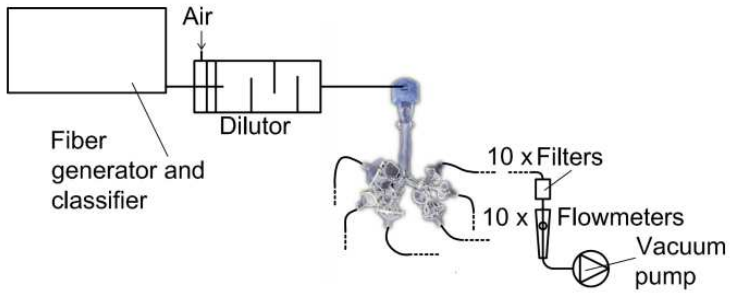

Figure 1 The simplified scheme of the experimental setup.

The experimental setup (**Fig 1**) consists of a fiber generation system, a classifier, a humidifier, a dilutor, the model of lungs with ten outputs, ten filters and flowmeters (each connected to one model output), and a vacuum pump. The complete description of the fiber generation and classification system was published by Wang et al [5]. Concisely, the glass fibers with the average diameter 1 μm and density 2.56 g/cm^3 were dispersed to the air, humidified, their electrical charge was removed by ^{210}Po ionizing unit and they were classified according to their length. The fibers coming out from the classifier were monodispersed in length with mean length 10 μm. They were mixed with an air in the dilutor to achieve homogenous aerosol with flowrate 30 l/min, which corresponds to steady inspiration during deep breathing. The inner surface of the model was coated by silicone oil to simulate mucous layer and to prevent bouncing of fibers from the wall. The aerosol flew through the model and non-depositing particles were collected behind the model outputs on nitrocellulose membrane filters. The flow through the outputs of the model was controlled by flowmeter valves to simulate realistic conditions.

Subsequent analysis of fiber deposition was based on modified PCM (Phase-Contrast Microscopy) method [6]. The model was disassembled into segments and each segment was put into a beaker with isopropanol and sonicated to release the fibers into the solvent. Isopropanol with fibers was then filtrated through nitrocellulose filters. Filters with deposited fibers were dried in a dustless environment. After that the filters were rendered transparent using acetone vapors and mounted on a microscope slide. Phase-contrast microscopy enables visualization of transparent glass fibers deposited on a transparent filter and mounted on a transparent microscope slide due to phase shift of the light passing through a fiber in a sample. The phase shift is visualized using interference with a non-shifted reference beam.

High resolution gray scale imaging of the filters was made using a camera Atik 320 E mounted on a microscope with phase contrast (Nikon Eclipse), and novel software was developed for identification and counting of the fibers in images.

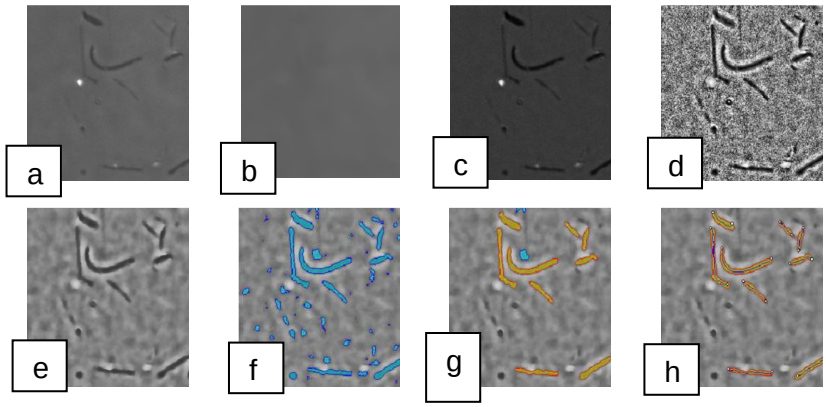

Figure 2 Procedure of the automated analysis: a) original image, b) background, c) calibrated image, d) application of Adaptive Contrast Control method, e) rotating Gaussian kernel, f) threshold method segmentation, g) removal of non-fiber objects, h) analysis of fiber ending

The image analysis consists of six main steps; results of each step are presented in **Fig 2**. The original image (**Fig 2a**) is down-scaled, a median filter is applied on the result to remove the fibers, and then the image is rescaled back to the original resolution using iterative bilinear interpolation with the scale factor 1.5 (**Fig 2b**) to create an artificial background image. The calibration is performed by dividing the original image by the image of the background (**Fig 2c**). The next step is the Adaptive Contrast Control method (ACC) which enhances fine details (**Fig 2d**). The noise reduction is done by means of a linear filter with rotating Gaussian kernel (**Fig 2e**). The following image shows the image being split in the identified objects and the background (**Fig 2f**). The fibers differ from other objects in size and shape. First, small objects are removed. Then, shape of the potential fibers is analyzed and objects that are not elongated enough are removed (**Fig 2g**). The last step is the analysis of identified fibers (**Fig 2h**).

3 Results and discussion

Two experiments for steady inhalation regime with flowrate 30 l/min are presented in **Fig 3**. Stokes number used for comparison of different particles in different lung models was calculated on the basis of aerodynamic diameter according to [3]

$$d_{ae} = d_{ve}\sqrt{\rho/(\rho_0 \kappa_r)} \tag{1}$$

where d_{ve} is volume equivalent diameter, ρ is the density of fiber, ρ_0 is the density of water and κ_r is the dynamic shape factor for randomly oriented prolate spheroid.

The Stokes number is then given by

$$\mathrm{Stk} = \frac{\rho_0 d_{ae}^2 U_s}{18\eta D} \tag{2}$$

where U_s is the mean velocity in the parent tube of the bifurcation, η is the air viscosity and D is the mean diameter of the parent tube of the bifurcation.

Deposition efficiency is a ratio of the number of fibers deposited in a current segment to the number of fibers entering the segment. Comparison of our data for fibers with previously published deposition of spherical particles (**Fig. 3**) demonstrated that the deposition of fibers is lower than the deposition of spherical particles. Therefore fibers can penetrate deeper into the lungs.

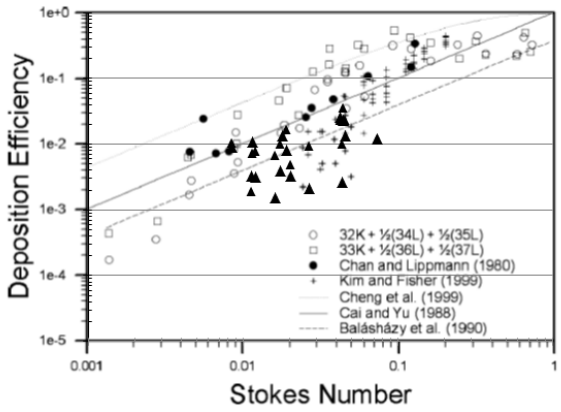

Figure 3 Comparison of fiber with spherical particles deposition.
Current data are represented by black triangles. (Modified from [8])

4 Summary

A novel approach was applied for evaluation of fiber deposition in the model of human lungs. The glass fibers were rinsed from the model and deposited on nitrocellulose membrane filters. Filters were rendered transparent and scanned by high-resolution camera. Images were then processed by special algorithms and deposited fiber counts were exported. Calculated deposition efficiency was compared to previously published data for spherical particles. Lower deposition of fibers in comparison to spherical particles is induced by their tendency to orient themselves into the flow direction. Future experiments will be focused on the influence of different breathing regimes and different lengths of fibers.

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