7 Measurement of Regional Alveolar Ventilation With FMS Aerosols

Alveolar deposition of appropriately sized polystyrene FMS labels shows promise as a means to obtain high-resolution maps of regional ventilation. While larger particles deposit on the conducting airways, an aerosol of ≥ 1.0 micrometer particles will deposit primarily in the alveoli rather than on conducting airways, and the intensity of the fluorescence deposited is proportional to the regional alveolar ventilation.

While the smallest possible particle size would best reflect the movement of gas within the lung, the size of the fluorescent signal for a given microsphere is proportional to the cube of the particle diameter, and a balance between these two constraints must be sought. We have found that we can obtain a satisfactory fluorescence signal from lung regions of 1.0 cm^3 volume with 1.0 micron FMS. Gas movement to lung regions of 1.0 cm^3 volume comes primarily by convection, and hence the FMS aerosol method is appropriate at that scale. However it would be inappropriate to do microscopic counts of 1.0 micron FMS deposition and conclude that differences in particle deposition between adjacent alveolar clusters represented differences in regional ventilation. Gas molecules equilibrate within alveolar spaces by diffusion, whereas aerosol particles of the 1.0 micron size deposit by gravitational settling. Microscopic examination of lungs given 1.0 micron FMS aerosol exposure reveal a heterogeneous deposition pattern in alveolar spaces, and a suggestion that most particles are deposited on the same side of the airway walls, as would be expected for gravitational settling. Microscopic examination of these samples does reveal that there is only rare FMS deposition on surfaces of the conducting airways, mostly at airway bifurcations. More recently, we have found that adequate fluorescent signal may be obtained using a 0.04 micron FMS administered over a 10-min period. The deposition of a 0.04 micron particle will be substantially greater than that of a 1 micron particle thereby partially compensating for the decreased amount of dye loaded into each microsphere.

Aerosol Administration System

The generation system should supply a stable monodisperse aerosol at a constant concentration throughout the administration period. It is desirable to be able to switch readily to different color labels. While the system described below attains these goals, it is possible that far simpler systems could work equally well if their aerosol composition were validated.

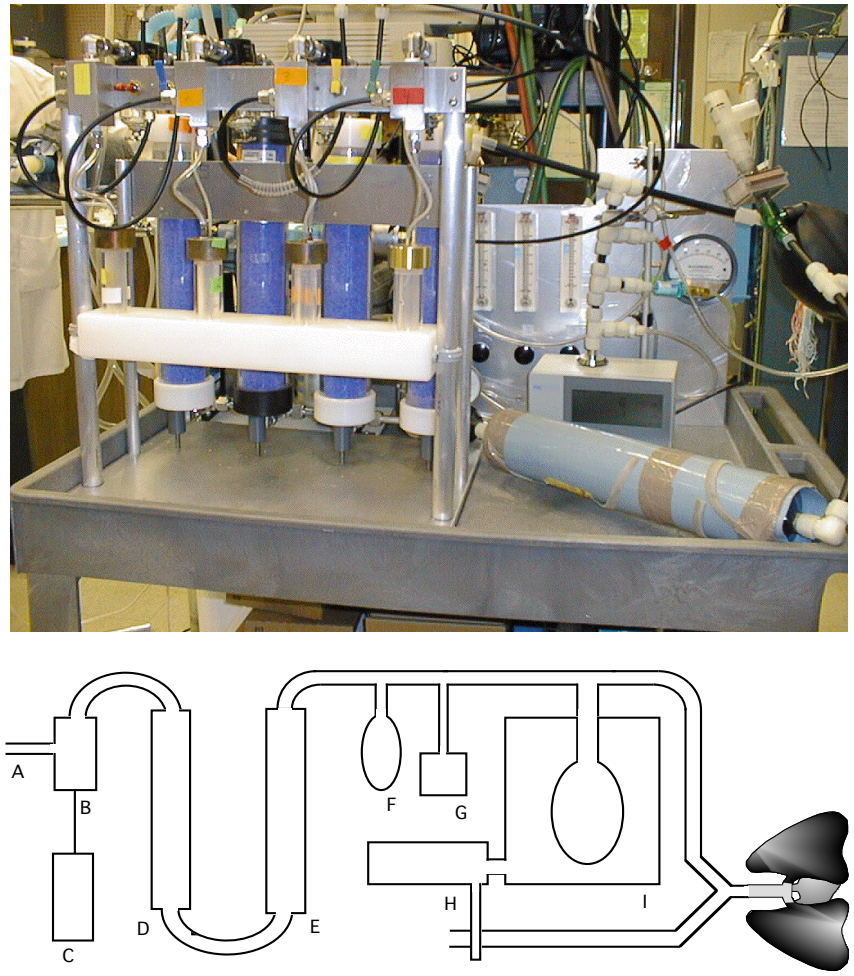

Figure 1. Current aerosol administration system with a schematic diagram.

1 The aerosol generator (B) draws the FMS suspension from the aerosol reservoir (C). We use a 3% suspension of FMS in distilled water with 0.02% Tween-80 and 0.01% thimerosal that is first vortexed and then sonicated for 30 seconds before pouring the mixture into the atomizer reservoir (C).

2. The aerosol is passed through a silica gel diffusion drier (D) to absorb the water, and through a Krypton-85 source charge neutralizer (E) to minimize deposition of charged aerosol on the walls of the administration system.

3. The aerosol has additional gas added to the reservoir bag (F) to satisfy the minute ventilation requirements of the animal, and the administered particle concentration is monitored with a laser particle counter (G).

4. The gas-aerosol mixture is administered to the lungs by a piston-pump ventilator (H) that drives a bag-in-box ventilation system (I). The aerosol only contacts conductive tubing and anesthesia bags with this ventilator, minimizing the loss due to electrostatic attraction.

Approaches to Maximizing the FMS Signal

Several different approaches to maximizing the FMS aerosol signal delivered to the lung may be used, depending on the constraints of the experimental protocol. The first is to minimize the loss of aerosol within the administration apparatus itself. We found that feeding the aerosol into a standard piston-pump ventilator consumed 2/3 of the generated aerosol. The bag-in-box system described above consumes very little of the administered dose. Higher signals are obtained when the manufacturer maximizes the amount of fluorescence loaded on the microspheres. The concentration of fluorescence added to microspheres for cell biology applications is substantially lower than can be attained. The choice of fluorescent marker is important if signals are low-several dyes have very high specific fluorescence relative to other colors. Larger microspheres contain more dye, and hence have a greater signal, but particles larger than 2.0 microns will have significant deposition on the conducting airways. A higher density of FMS can be added to the aerosol apparatus, provided the aerosol generated remains monodisperse. Finally, if the experimental protocol permits, increasing the FMS administration time or altering the ventilatory parameters to increase settling time (larger tidal volumes or adding an inspiratory hold) will increase deposition.

Potential Problems with the Aerosol Administration

Bacterial contamination of the FMS solution can occur, particularly if FMS suspensions are recycled from previous experiments. Aerosolized bacteria from such suspensions produce pulmonary hypertension, systemic hypotension, and hypoxemia in experimental animals. Cleaning and drying the generating system after each use and using thimerosal in the FMS suspension appears adequate to prevent this problem.

Deposition of particulates in the lung is not the same as retention, as particles deposited on ciliated airways will eventually be cleared over a period of hours. Particles deposited in alveolar spaces must be cleared by pulmonary macrophages, which then migrate to the airways. The latter process requires days. All of our studies have lasted less than 90 minutes before the lungs were removed and air-dried. We have not demonstrated any difference between particle distributions administered 90 minutes apart (see below), and would not expect a difference for particles deposited primarily in alveolar spaces.

The FMS mixture is supplied in distilled water with 0.01% thimerosal and 0.02% Tween-80. The distilled water is required to permit the microspheres to dry without a saline coating as they are administered. Adsorbed saline will cause the microspheres to pick up water and grow in size once they are in the fully humidified environment of the pulmonary airways.

Potential Problems with Analysis of FMS Aerosol Signals

The small FMS have a far greater surface to volume ratio in comparison to the standard 15 micron FMS used for blood flow studies, and are prone to decolorization artifacts that cannot be detected with the larger microspheres. We have demonstrated that if a TLC-dried lung labeled with a 1.0 FMS aerosol is foamed with the quick setting isocyanate foam used with our previous studies, fluorescent activity is lost at the lung periphery, particularly for the yellow-green and blue-green fluorescent labels. The simple solution to this problem is to first coat the lung with a 1 to 2 cm layer of a slow setting polyurethane foam (Kwik Foam, DAP Inc., Dayton OH).

Because of the smaller fluorescence signal, two potential problems are background fluorescence from the lung and spillover from dyes adjacent to the ventilation marker in the fluorescent spectrum. Background fluorescence is most marked on the blue end of the spectrum, so that the colors blue, blue-green, and yellow-green are most influenced. A second important factor for lung samples is the amount of light scattering produced by a high particulate concentration in the soaked lung sample. A deliberately macerated piece of lung without fluorescence added can produce a background signal that can be eight times above the background signal for an intact, cleanly cut piece of lung. This artifact will resolve if the particles are allowed to settle in the cuvette, but adequate settling may require several minutes. Signals may be corrected for spillover from adjacent fluorescent colors using a matrix inversion technique (see Section4). Since signals from intravenously delivered 15 micron microspheres tend to be much larger than ventilation signals, we choose fluorescent colors that are adjacent on the fluorescent spectrum for our ventilation markers.

Simultaneous administration of different FMS markers has revealed that a small fraction (less than 1%) of the ventilation measurements show marked discrepancies, with one measurement being several standard deviations higher than its companion. Similar high ventilation signals in those particular pieces are never observed with any measurements preceding or following that measurement. We presume that these high signals are artifacts. While this artifact has been observed with all FMS colors, it occurs most frequently with orange. The two explanations which seem most likely are either there are very rare large "chunks" of color which do not deposit out either in the administration system or the conducting airways, or there are some naturally inhaled substances which give fluorescence. Favoring the former explanation, microscopic examination of FMS suspensions at low power will rarely reveal large irregular chunks of color among the uniformly sized microspheres.