



Studies of Radioaerosol Deposition in the Respiratory Tract

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Deposition of aerosols in the respiratory tract can be quantitatively and qualitatively studied by scintigraphy. The most commonly used radionuclide for this purpose is technetium-99m. The effects of various factors on particle deposition have been investigated by using radio-labeled aerosols in the past decade. Most of these studies were *in vivo* but some were *in vitro* or *ex vivo*. The factors examined include particle size, formulation, inhaler design, inhalation flowrate, body posture, and gravity. They have been shown to influence pulmonary deposition, nasal high flow nebulization, and intranasal delivery. A thorough understanding of the various factors is required for the advancement of respiratory-drug delivery. Scintigraphy is a powerful technique that can assist in this regard.
Semin Nucl Med 49:62-70 © 2018 Published by Elsevier Inc.

Introduction

Radiolabeled aerosols have long been used to image, track, and quantify pharmaceutical aerosol particle deposition in the respiratory tract *in vivo*. They are commonly employed in dosing and/or pharmacokinetic studies, as well as in investigations on physiological responses to inhaled aerosols. Technetium-99m (^{99m}Tc) is the most commonly used gamma-emitting radionuclide due to its relatively short half-life (6.02 h), low cost, and simple production method.¹ High quality images are obtained by detecting its high energy photons (140 keV) with a gamma camera. Pharmaceutical inhalation formulations such as dry powder inhalers (DPIs), metered dose inhalers (MDIs), soft mist inhalers (SMIs), and nebulizers have been radiolabeled successfully. However, most *in vivo* deposition studies use nebulizers because mixing the radiolabel with the liquid formulation is easy and no drying is required.

With the advancement of scintigraphy technology, there is an increase in number of studies that examine the influence of various factors on pulmonary deposition, nasal high flow nebulization, and intranasal delivery *in vivo*. These factors can be related to the product properties (aerodynamic

particle diameter, formulation, and inhaler) or patient factors (inhalation flow rate and/or pattern and posture). This paper provides a review of the scintigraphy studies on the effects of the various factors reported over the past decade.

Pulmonary Deposition

Influence of Particle Size

Aerodynamic diameter is the most important parameter that affects particle deposition. It is well-known that the dominant deposition mechanism for particles with aerodynamic diameters of $>5 \mu\text{m}$, $1\text{--}5 \mu\text{m}$, and $<1 \mu\text{m}$ are inertial impaction, gravitational sedimentation, and diffusion, respectively.² The aerodynamic diameter of aerosolized particles is conventionally measured *in vitro* by cascade impaction. The fine particle fraction, commonly defined as the percentage of particles with aerodynamic diameters $<5 \mu\text{m}$ in the loaded, recovered, or emitted dose, can also be derived from this measurement. Although the effect of particle size on aerosol performance has long been demonstrated *in vitro*, it has only been studied systematically *in vivo* in the past decade.

Radiolabeled particles or droplets of various sizes are produced by incorporating the radionuclide into the powder or liquid formulation before aerosolization. Particle size can be controlled by varying the process parameters or devices for aerosol generation. Radiolabeled mannitol particles with volume median diameters of 2, 3, and $4 \mu\text{m}$ (Fig. 1) were produced by spray drying aqueous mannitol solutions

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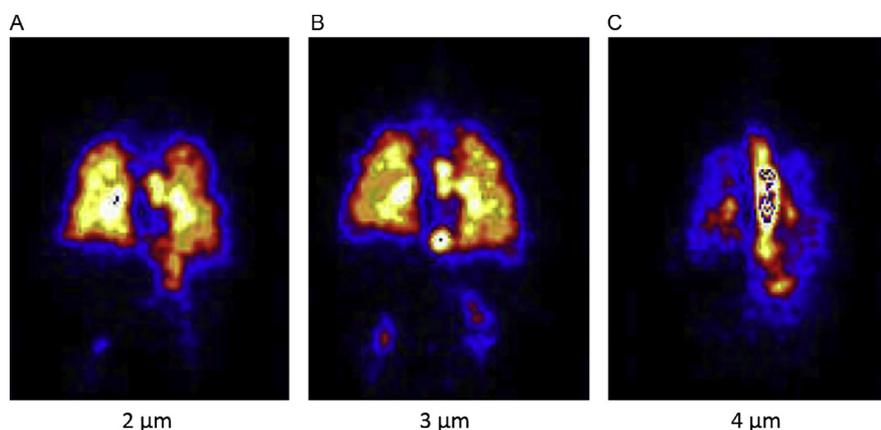


Figure 1 Representative SPECT coronal slicing images from the same subject after inhaling radiolabeled mannitol particles with volume median diameters of (A) 2 μm , (B) 3 μm , and (C) 4 μm . Reproduced with permission from Glover et al.³

containing 4 GBq of $^{99\text{m}}\text{Tc}$ complexed with diethylenetriaminepentaacetic acid (DTPA).³ The concentration of dissolved mannitol, liquid feed rate, and atomising nozzle airflow were varied to control the particle size.³ The radioactivity of the mannitol powders was 1-1.5 GBq/g, with the [$^{99\text{m}}\text{Tc}$] DTPA complex remaining intact after spray drying. The corresponding aerodynamic diameters and geometric standard deviations (GSD) were 2.7, 3.6, and 5.4 μm , and 2.6, 2.4, and 2.7, respectively, when the powders were dispersed from an Aeroliser inhaler into a Marple Miller Impactor at both 60 and 100 L/min. As expected, the *in vitro* FPF increased with decreasing particle size. For the *in vivo* study, three capsules containing 20 mg of radiolabeled mannitol per capsule were inhaled with maximum respiratory effort from an Aeroliser by healthy adult subjects. The delivered dose and particle distribution in the lungs were then measured using SPECT. The amount of exhaled radiolabeled aerosol was minimal for all three particle sizes. The lung dose, indicated by the total radioactivity in the lungs, increased with decreasing particle size. In particular, the lung dose of the largest particles (20.6% of the loaded dose) was about half of that of the medium-sized and smallest particles (38.9% and 44.7% of the loaded dose, respectively).³ This trend was also shown in the penetration index (PI), which was the ratio of the counts per voxel in the peripheral lung region to those in the central region (PI = 0.52, 0.60, and 0.63 for the 5.4, 3.6, and 2.7 μm particles, respectively). This indicated that the smaller the particles, the more peripheral the deposition (ie, deeper penetration). On the other hand, extrathoracic deposition, calculated as the total radioactivity in the oropharynx and gastrointestinal tract, showed the opposite effect. About 75.9% of the loaded dose deposited in the upper airways for the largest particles, while it was 42.4% and 36.0% for the medium-sized and smallest particles, respectively.³

Similar findings were reported by other research groups. In a study by Majoral et al, healthy adult subjects inhaled aerosols containing $^{99\text{m}}\text{Tc}$ -labeled human serum albumin in isotonic saline generated from an AKITA² device that was connected to a PARI vibrating mesh nebulizer.⁴ The AKITA² device was set to produce droplets with volume median

diameters of about 3 or 6 μm . Measurements were performed by two-dimensional (2D) planar imaging and three-dimensional (3D) SPECT coupled with CT. The large droplets deposited more centrally and showed higher extrathoracic deposition and 24-hour clearance than the small ones. On the other hand, the small droplets achieved higher total airway deposition.⁴ The radioactivity in the left lung was lower than that in the right lung irrespective of particle size, with a mean left-to-right deposition ratio of 0.87. This was due to the smaller volume of the left lung. However, when the radioactivity in each side was normalized by the corresponding lung volume, the mean ratio became 1.08. Thus deposition was slightly higher in the left lung relative to its volume. The left lung also showed more deposition in the central airways and higher 24-hour clearance than the right lung.⁴ This implies that particle distribution was asymmetrical between the two lungs, with lower particle penetration in the left. In a study reported in 2018 employing $^{99\text{m}}\text{Tc}$ -labeled salbutamol in idiopathic pulmonary fibrosis patients who were over 40 years old, monodisperse particles (GSD <1.22) of 1.5 and 6 μm produced from a spinning-top aerosol generator were compared with polydisperse particles (GSD >1.22) of an unspecified size generated from a PARI BOY SX jet nebulizer.⁵ The monodisperse 1.5 μm particles showed higher total lung deposition than the monodisperse 6 μm particles and polydisperse nebulized particles (64.9%, 50.5%, and 8.19%, respectively). The nebulizer showed poor delivery efficiency not only from the low lung dose of 8.19%. Most of its dose (85.2%) was actually retained in the device itself and not emitted. The 6 μm particles deposited more in the throat than the 1.5 μm particles and nebulized particles (30.2%, 10.8%, and 5.1%). However, the 1.5 μm particles were most easily exhaled, due to its small size.⁵ The 1.5 μm particles and polydisperse nebulized particles showed much higher pulmonary penetration (PI = 0.8 and 0.79, respectively) than that of the 6 μm particles (PI = 0.49). Interestingly, particle penetration in the left and right lungs was largely similar for a given particle size in this study, unlike the asymmetry observed by Majoral et al.⁴ However, the PI was found to decrease with a reduction in the forced vital capacity of the patient, signifying an increase

in the severity of idiopathic pulmonary fibrosis.⁵ Besides in vivo human scintigraphy studies, the size-dependency of particle deposition have also been investigated ex vivo in a lung model⁶ and in vivo in nonhuman primates.^{7,8} The ex vivo model was composed of a plastinated human head connected by plastic tubes to a pair of porcine lungs.⁶ The lungs were enclosed in a sealed enclosure that generated the “inhalation” and “exhalation” by expanding the enclosure and return it to its resting state, respectively, thereby mimicking the action of the pleural cavity during breathing. A 4 mL solution containing 740 MBq of [^{99m}Tc]DTPA was nebulized from three jet nebulizers (Atomiser NL11, modified Side-stream, Nanoneb) coupled to an AOHBOX air compressor operating at 6 L/min.⁶ The activity median aerodynamic diameter and GSD of the particles generated were 2.80 μm , 550 nm, and 230 nm and 3.2, 2.1, and 1.6, respectively. The aerosols were delivered into the lung model via a nasal plug under simulated tidal breathing of an adult male. Deposition was assessed using 2D and 3D SPECT/CT. The exhaled fraction of the small, medium, and large particles was 68%, 34%, and 27%, respectively.⁶ This agrees with the notion that submicron particles cannot deposit efficiently in the lungs during normal breathing as there is insufficient time for them to deposit by diffusion. As expected, extrathoracic deposition increased and thoracic deposition decreased with particle size. Most of the dose that deposited in the lungs for all three particle sizes was in the peripheral region, with < 5% of the thoracic dose in the central region.⁶

The effect of particle size on lung deposition have been explored on nonhuman primates such as cynomolgus monkeys (*Macaca fascicularis*)⁷ and rhesus macaques (*Macaca mulatta*)⁸ because they are common animal models for studying respiratory infections. Aerosols containing ^{99m}Tc-labeled Dulbecco's Eagle's minimum essential medium supplemented with foetal bovine serum and cell lysate solution were generated using a six-jet Collison nebulizer and a Retic nebulizer.⁷ The activity median aerodynamic diameter and GSD of the nebulized particles were 2.3 and 5.1 μm , and 2.7 and 1.9, respectively. The aerosols were administered to anesthetized cynomolgus monkeys via a nasal mask under tidal breathing for five minutes, followed by gamma scintigraphy. Although the total deposition of the smaller particles in the respiratory tract was lower than that of the larger particles (51.2% vs 65.9%), they achieved higher lung deposition and lower extrathoracic deposition (12.3% vs 7.8% and 39.0% vs 58.1%, respectively).⁷ In another study, anesthetized rhesus macaques inhaled aerosols containing fluorodeoxyglucose ([¹⁸F]FDG) as the radiolabel generated from an ultrasonic nozzle via an oronasal mask under tidal breathing for 15 minutes. The particle size distribution was varied by changing the solute content in the solution by diluting the radiolabel stock with water and/or Luria Bertani Lennox broth containing 4% v/v glycerol. The particles produced ranged from 1.7 to 11.8 μm in mass median aerodynamic diameter (MMAD) and were relatively monodisperse, with GSDs from 1.18 to 1.25. Results from PET/CT showed that deposition in the lungs decreased with particle size (24.1% and 0.1% for 1.7 μm and 11.8 μm particles, respectively).

On the other hand, deposition in the oronasal region increased with particle size (30.2% and 80.1% for 1.7 μm and 11.8 μm particles, respectively). Thus the scintigraphy data discussed above provide direct evidence of the inverse relationship between particle size and deposition. The same trends were seen in vivo in humans and monkeys, as well as ex vivo in a porcine lung model.

Influence of Formulation

The type and constitution of the formulation can affect particle deposition through influencing particle size and other aerosol characteristics such as particle velocity and plume duration. The most obvious example is the difference in the size of the particles generated from solution and suspension MDIs, with the former producing significantly smaller particles.⁹ MDIs with hydrofluoroalkane (HFA)-134a as the propellant, QVAR (solution; 40 μg beclomethasone dipropionate per puff) and Flovent (suspension; 44 μg fluticasone propionate per puff), have been radiolabeled with pertechnetate (TcO_4^-) and compared in vivo in adult asthmatic patients.¹⁰ SPECT/CT were employed to obtain 2D and 3D data. The MMAD and GSD of QVAR and Flovent were 0.7 and 2.0 μm and 1.7 and 1.6, respectively.¹⁰ Thus, particles from the solution MDI were nearly three times smaller than those from the suspension MDI. Consequently, QVAR achieved higher lung deposition (55% vs 24%) and lower oropharyngeal + gut deposition (45% vs 75%) than Flovent.¹⁰ Moreover, 31.9% of the lung dose for QVAR was in the peripheral region, whereas it was 12.3% for Flovent, because the smaller particles penetrated deeper into the lungs. Although the exhaled radioactivity was low for both MDIs, it was slightly higher for QVAR due to its submicron particles. A similar study conducted by the same research group with HFA QVAR and HFA Advair (suspension; 115 μg fluticasone propionate and 21 μg salmeterol per puff; MMAD 2.7 μm) reported similar trends as those discussed for HFA Flovent.¹¹

A major difficulty with using MDIs is to coordinate actuation and inhalation. Another problem with MDI aerosols is that the emitted particles travel at high velocity (~80 km/h), resulting in high impaction in the throat. A novel breath-synchronized MDI called the Tempo inhaler has been designed to overcome both problems.¹² It has mechanical features that release the aerosol upon an inhalation trigger and reduce the plume velocity by about 90%. The dose release trigger can also be adjusted to be at the early or later part of the inhalation so that deposition can target the peripheral or central airways, respectively.¹² The deposition of particles from Tempo and Flovent inhalers, both chlorofluorocarbon-based with 110 μg fluticasone per puff, were compared in healthy adults using gamma scintigraphy. The maximum dose of radioactivity administered was 10 MBq per experiment. The Tempo inhaler showed higher whole lung deposition (41.5% vs 13.8%) and lower oropharyngeal deposition (18.3% vs 76.8%) than Flovent.¹² Moreover, deposition in all the pulmonary regions (ie, central, intermediate, and peripheral) were

about three times higher for the Tempo inhaler than for Flovent. Exhaled radioactivity was low for both inhalers but was higher for Tempo (1.5% vs 0.5%).¹²

In vivo deposition of aerosols from MDIs has been compared with those from SMIs¹³ and nebulizers.¹⁴ SMIs produce aerosols by mechanically colliding two streams of the liquid formulation to form droplets, without a propellant. The plumes are slower and last longer than those from MDIs to supposedly reduce oropharyngeal deposition. A Respimat SMI and an HFA-based MDI containing the same ^{99m}Tc-labeled Berodual formulation (50 µg fenoterol hydrobromide and 20 µg ipratropium bromide per puff) were compared by gamma scintigraphy in chronic obstructive pulmonary disease patients before and after training on the use of the inhalers.¹³ The maximum dose of radioactivity administered was 4 MBq per day. The SMI showed higher whole lung deposition than the MDI for both untrained (37% of delivered dose vs 21% of metered dose) and trained patients (53% of delivered dose vs 21% of metered dose). The SMI showed the same oropharyngeal deposition as the MDI before training (56% vs 56%) but it was reduced with training (45% vs 55%).¹³ It should be noted that the whole lung and oropharyngeal depositions of the MDI were unchanged with training. Deposition in the central, intermediate, and peripheral lungs regions were all higher for the SMI. The PI was also higher for the SMI than the MDI (0.45-0.55 vs 0.39-0.44), indicating deeper deposition.¹³ Particle deposition between an MDI and a jet nebulizer was compared in vivo in foxhounds.¹⁴ Flixotide MDI (250 µg fluticasone propionate per puff) was radiolabeled with sodium pertechnetate. Each dog received two actuations from this MDI via a Breath-A-Tech spacer and mask. The dogs took five tidal breaths after each actuation.¹⁴ On the other hand, commercial fluticasone propionate nebulizers were radiolabeled with [^{99m}Tc]DTPA so that 1.5 mL of the solution contained 200 µg of the drug and 100 MBq of activity. The nebulized aerosol was delivered to the dogs using an Econ-o-mist Forte Nebulizer and a mask under tidal breathing for one minute. The data from 2D gamma scintigraphy showed that nebulization gave higher respiratory tract deposition (4.2% vs 2.3%) but also higher extrathoracic deposition (50.0% vs 5.3%) than the MDI.¹⁴ The radioactivity retained in the device setup (eg, mask, connectors, MDI actuator, and spacer) was lower for the nebulizer than the MDI (13.9% vs 36.9%). This was due to the higher particle deposition in the spacer by impaction and sedimentation. Whether the dogs were sedated or not during aerosol administration did not affect respiratory tract deposition.¹⁴

Influence of Inhaler Design and Inhalation Flowrate

The type and design of the inhaler can affect deposition through influencing not only the airflow and dispersion characteristics, but also the geometry of the upper respiratory tract during inhalation. These effects are especially prominent for DPIs because they are passive inhalers that solely

rely on the patient's inspiratory effort for aerosolization. There were studies that examined the effect of flowrates with the same DPI. (ie, same resistance) from the 1990s to the first half of the 2010s. However, although lung deposition was shown to increase with flowrate for some DPIs (Spinhaler (air resistance = 0.016 kPa^{1/2} min/L), Turbuhaler (0.039 kPa^{1/2} min/L), and Inhalator (0.062 kPa^{1/2} min/L)), it was independent of flowrate for other high resistance DPIs (Pulvinal, Air, and Taifun).¹⁵ Thus, the effect of flowrate on deposition is inhaler-specific and cannot be simply explained by the numerical value of inhaler resistance. Glover et al found that the in vitro FPF and in vivo lung dose were independent of flowrate with the Aerolizer (0.019 kPa^{1/2} min/L), which is a low resistance DPI.³ The in vitro flowrates for cascade impaction were 60 and 100 L/min, while the in vivo peak inhalation flowrates (PIFR) achieved by the subjects were all >90 L/min. The lack of flow-dependence was attributed to the establishment of a maximum FPF at 60 L/min due to the generation of a critical turbulence level within the Aerolizer at this flowrate for powder dispersion.³ Therefore, the FPF plateaued at flowrates ≥60 L/min.

Another study examined the effect of DPI resistance and flowrate using the Osmohaler.¹⁵ The oropharyngeal cavity can partially collapse and narrow when inhaling through a DPI to generate the pressure drop. The higher the inhaler resistance, the more the oropharyngeal cavity narrows to generate the same flowrate. The resultant change in the upper airway geometry can affect the airflow pattern in that region, consequently affecting particle deposition there and downstream. The deposition of spray dried mannitol particles (volume median diameter = 3.3 µm) radiolabelled with [^{99m}Tc]DTPA was measured in healthy human adults using low resistance (0.021 kPa^{1/2} min/L) and high resistance (0.036 kPa^{1/2} min/L) Osmohalers at PIFRs of 50-70 L/min.¹⁵ Two capsules containing 30 mg radiolabeled mannitol with a total radioactivity up to 120 MBq were inhaled by each subject, followed by SPECT imaging. Total lung deposition was not significantly affected by the resistance (19.9% and 17.5% for low and high resistance, respectively) but it decreased to 9.8% when the low resistance Osmohaler was used at PIFRs of 110-130 L/min. The in vitro FPF (30.8% vs 16.4%) also decreased for this low resistance DPI when the flowrate increased from 65 to 120 L/min on a Multi-Stage Liquid Impinger with an Alberta Idealised Throat.¹⁵ Total lung depositions showed positive correlation with in vitro FPF (Fig. 2). This indicated that dispersion from the Osmohaler depended on particle size and flow rate, but not resistance. This is because both a larger particle size and higher flow rate increase extrathoracic deposition, hence reducing deposition in the lower airways. Most of the particles deposited in the middle region of the lung, regardless of resistance and flow rate.¹⁵ However, the PI showed closer association with the MMAD than the FPF (both measured in vitro with a Multi-Stage Liquid Impinger and an idealised throat), with the PI decreasing with increasing MMAD (Fig. 3). The highest reduction in the PI was observed when both the particle size and flowrate were increased. This led to an increase in the Stokes and Reynold numbers, which consequently

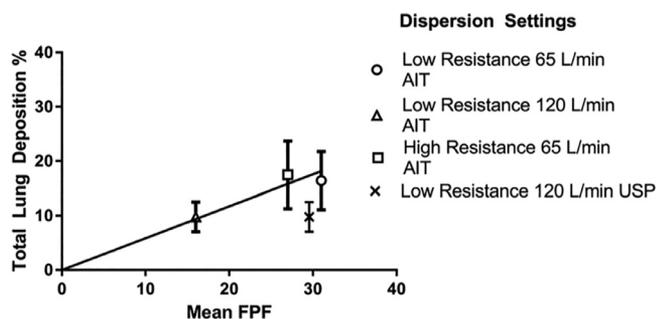


Figure 2 Correlation between in vivo total lung deposition and in vitro fine particle fraction. Reproduced with permission from Yang et al.¹⁵

increased inertial impaction in the central pulmonary region and reduced deposition by sedimentation in the periphery.¹⁵

The effect of flowrate on aerosol deposition was also observed in vitro in a 3D-printed bronchial cast derived from a healthy young female's CT scan and connected to an averaged upper airway model.¹⁶ A spinning-top aerosol generator generated monodisperse particles (GSD <1.22) of 6 μm aerodynamic diameter that were sampled into the in vitro lung model at 30 and 60 L/min by vacuum suction. Scintigraphic imaging was performed in two perpendicular planes, that is, frontal and lateral views. Total aerosol deposition increased with flowrate (28% vs 69% at 30 and 60 L/min, respectively).¹⁶ Most of the particles deposited in the first five airway generations, as expected from the relatively large aerodynamic particle size. Deposition biased toward the left lung at 30 L/min, with a left-to-right ratio of 1.35. This ratio was reduced to 1.16 when the flowrate was increased to 60 L/min.¹⁶ Although deposition biased toward the left lung, this side of the lung actually received a lower proportion of the total aerosol mass (sum of deposited and suspended particles) than the right lung. This was attributed to the lower gas ventilation in the left lung. Aerosol distribution in the five lobes of the lungs also followed the proportion of gas distribution in those lobes.¹⁶ The asymmetrical distributions

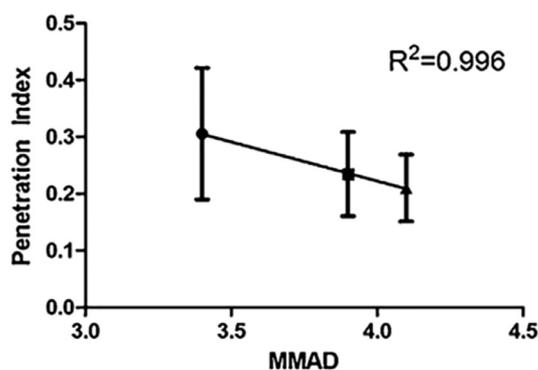


Figure 3 Correlation between penetration index and mass median aerodynamic diameter. Low resistance Osmohaler at 120 L/min (▲), high resistance Osmohaler at 65 L/min (■), low resistance Osmohaler at 65 L/min (●). Reproduced with permission from Yang et al.¹⁵

measured in this study were opposite to those discussed above from Majoral et al,⁴ who reported that the left lung had lower absolute deposition but it had higher deposition relative to its volume. Furthermore, Majoral et al⁴ found no difference in particle deposition between deep breathing (1 L tidal volume over 3.3 seconds) and shallow breathing (0.6 L tidal volume over 2.0 seconds) with the AKITA² device. It should be noted that this study was in vivo involving multiple subjects, whereas the one by Verbanck et al¹⁶ was in vitro using a 3D-printed bronchial model from one subject. The aerosols and inhalation schemes were also different between the two studies so the results are difficult to compare.

The lack of effect of the tidal breathing pattern on lung deposition was also observed in vitro by Laube et al¹⁷ using the Sophia Anatomical Infant Nose-Throat (SAINT) model. This 3D model was produced from the CT scans of the upper airways of a nine-month-old girl.¹⁸ Jet nebulized albuterol aerosols radiolabeled with ^{99m}Tc were sampled into the SAINT model at simulated tidal breathing. The breathing rate was 15 breaths/30 seconds. The duration of inhalation and exhalation was 0.9 second and 1.1 seconds, respectively. Three tidal volumes (50, 100, and 200 mL) were tested using this breathing scheme.¹⁷ Total lung deposition was not significantly different between the three tidal volumes, being 7.17%-9.41%. Aerosol deposition in the tubing and face mask was also comparable (11.42%-13.58%). However, deposition in the cavity increased significantly with tidal volume (4.4%, 11.39%, and 22.12% for 50, 100, and 200 mL, respectively).¹⁷ This was probably because a higher tidal volume meant faster airflow (since the duration of the breathing cycle remained constant), thus leading to more particle impaction in the nasal cavity during inhalation.

The importance of the mode of breathing to facilitate particle deposition has been demonstrated in a scintigraphy study on pediatric asthma patients.¹⁹ Budesol, a budesonide nebulizer, was radiolabeled with [^{99m}Tc]DTPA and nebulized by a modified PARI e-Flow Baby vibrating mesh nebulizer. The median mass diameter of the droplets was 2.6 μm . Each child sat or was held upright while inhaling a maximum dose of 2 MBq through a tight-fitting face mask, followed by gamma scintigraphy.¹⁹ The overall lung deposition was 34.3% but the mean lung deposition in the children who cried during aerosol administration was significantly lower than that in children who inhaled quietly (20.0% vs 48.6%, respectively). The mean lung-to-oropharyngeal deposition ratio was also much lower in crying children (0.27 vs 1.00).¹⁹ This indicates that the erratic breathing during crying hindered deposition in the lower airways and increased oropharyngeal deposition.

Controlled inhalation may be employed for targeting the deposition of very large particles. Normally at tidal breathing during nebulization (0.4 L tidal volume at 0.4 L/s), respirable particles are in the range of 1-5 μm in aerodynamic diameter.²⁰ Indeed, this is the droplet size range that most nebulizers generate. However, the deposition of particles larger >5 μm in small airways is possible if the flowrate is very low (eg ~0.05 L/s).²⁰ This is because extrathoracic impaction is reduced at such low flowrates. The transit time in the small

airways also becomes longer than sedimentation time. Therefore, the big particles can deposit deeper into the lungs. Healthy adult subjects inhaled about 40 μCi of $^{99\text{m}}\text{Tc}$ -labeled sulfur colloid particles in saline from a Devilbiss 646 jet nebulizer (5 μm MMAD, 2.0 GSD) and a Pari-Boy jet nebulizer with a modified baffle to produce larger droplets (9.5 μm MMAD, 1.8 GSD).²⁰ The subjects inhaled the 5 μm droplets with a mean tidal volume of 0.44 L at 0.46 L/s, whereas the 9.5 μm droplets were inhaled with a mean tidal volume of 0.8 L at 0.08 L/s. The conducting airway deposition was higher and the oropharyngeal deposition was lower for the 9.5 μm droplets at 0.08 L/s (35% and 26%, respectively) than the 5 μm droplets at tidal breathing (27% and 37%, respectively).²⁰ The use of large particles is advantageous if the drug is expensive or has much adverse effects because a larger mass can be delivered in a shorter time and with fewer inhalations. The targeting effect at very low flow-rates can also reduce dose wastage and improve drug localization in the airways.

Influence of Body Posture

Another patient factor besides flowrate is body posture during aerosol administration. The lungs can deform under its own weight so body posture can affect particle deposition through changing the geometry of, and ventilation in, the lungs. These differences may change the regional distribution of deposited particles. The effect of body posture in this regard was investigated in a couple of scintigraphic studies.

Healthy adults inhaled about 40 μCi of $^{99\text{m}}\text{Tc}$ -labeled sulfur colloid particles in saline via an Aeroneb vibrating mesh nebulizer in the supine and seated positions.²¹ The nebulized droplets had a MMAD and GSD of 4.9 μm and 2.5. The aerosols were inhaled tidally at 0.5 L/s at 15 breaths/min. Although the central-to-peripheral deposition ratios from the supine and seated positions were comparable (1.26 vs 1.43, respectively), there were differences in regional deposition.²¹ Relative deposition in the alveolar region increased from 34% in the supine position to 60% when seated. On the other hand, deposition in the intermediate (16% vs 7%) and small airways (34% vs 19%) were reduced when seated. The trends were attributed to the airways becoming narrower in the supine position.²¹ This increased the likelihood of impaction and sedimentation because particles travel less distance before deposition. Moreover, since the inhalation rate was constant, narrower airways will increase linear airflow velocity, which increases impaction.²¹ Thus more particles deposited in the intermediate and small airways rather than the alveolar region when the body was in the supine position.

Another study compared particle deposition between lying on the right and the left in the lateral decubitus position.²² Healthy adult subjects inhaled saline radiolabeled with [$^{99\text{m}}\text{Tc}$]DTPA and aerosolized from a Venticis II jet nebulizer. The droplet size distribution was not reported.

It was found that absolute deposition was higher in the right lung when the subject was lying on the right.²² Conversely, deposition was higher in the left lung when lying on

the left. This was clearly from the influence of gravity. The relative deposition pattern within each lung was similar, with most deposits in the intermediate and peripheral regions.²² Position-dependent particle deposition may offer therapeutic advantage in delivering drugs to a particular side of the lungs for local targeting.

Influence of Gravity

Gravitational sedimentation is the major deposition mechanism for particles in the inhalable size range (1-5 μm aerodynamic diameter). Besides affecting total deposition, gravity also influences regional deposition and retention. An interesting scintigraphic study conducted by Darquenne et al²³ examined the effect of microgravity (near-zero gravity) on particle deposition in vivo. The same experimental procedure as that by Sá et al²¹ mentioned above was conducted in a National Aeronautics and Space Administration Microgravity Research Aircraft for microgravity and on the ground for normal gravity.²³ The nebulized droplets had a MMAD and GSD of 5.6 μm and 2.4, respectively, under microgravity and 4.9 μm and 2.5 under normal gravity, respectively.²³ The central-to-peripheral deposition ratio was 1.57 and 1.28 under microgravity and normal gravity, respectively. Thus particles deposited more centrally under microgravity. This was because gravitational sedimentation is the main deposition mechanism in the deeper airways. Therefore, less particles would deposit there without gravity. These particles could then deposit in the central airways during exhalation. Hence the effect is compounded in central deposition. Moreover, there were more localized deposition "hot spots" lungs under microgravity, due to inertial impaction. While 58% of the total lung deposition was in the alveolar region under normal gravity, it was 17% under microgravity.²³ Conversely, while only 14% of the total lung deposition was in the large airways under normal gravity, it was 53% under microgravity. Particle retention in the whole lung and in the tracheobronchial region was also shorter under microgravity. The particles were cleared out quicker in the absence of gravity because more particles deposited in the central airways and consequently were removed by mucociliary clearance.²³ Although the findings from this study may not be directly relevant in the conventional clinical setting, it has implications on the deposition and clearance of inhaled (potentially harmful) particles for astronauts in space.

Nasal High Flow Nebulization

High-flow nasal cannula has been increasingly administered as supportive therapy in critical care to improve oxygenation in patients with respiratory failure. It delivers at high flow (up to 60 L/min) heated and humidified gas (37°C, 100% relative humidity) with a controlled oxygen concentration (21%-100%) via a nasal cannula.²⁴ Patients who are using a high-flow nasal cannula may also require aerosolized drugs, such as bronchodilators, to treat their condition. Thus nebulization has recently been combined with the nasal cannula

setup for this purpose. The delivered gas needs to flow through the nasal cavity before entering the lungs. Pulmonary drug delivery using this setup is more challenging than via the oropharynx as the transnasal route is longer and more tortuous. Particles may also be captured in the nasal cavity and cannot deposit further downstream.

The performance of a vibrating mesh nebulizer (Aerogen Solo) and a jet nebulizer (Opti-Mist Plus, operated at 8 L/min) coupled to a high-flow nasal cannula setup has been compared in healthy subjects.²⁵ The nebulizer was placed upstream of the humidifier in the circuit. The flow rate of the high-flow nasal cannula was adjusted to deliver 30 L/min of gas (37°C, 21% oxygen) when the nebulizers were in operation. A solution of [^{99m}Tc]DTPA in an unknown vehicle was nebulized continuously while the subjects inhaled tidally through the nose, followed by SPECT-CT.²⁵ Lung deposition was found to be very low, with only 3.6% and 1% of the nominal dose for the vibrating mesh and jet nebulizers, respectively. Substantial amounts of the dose deposited in the circuit, nasal cannula, upper respiratory tract, and lost to the environment. Deposition in the circuit plus nasal cannula for the vibrating mesh and jet nebulizers were 48.0% and 17.2%, respectively. The larger droplet size produced by the vibrating mesh nebulizer (MMAD 3.4 μm vs 1.8 μm from the jet nebulizer) could have caused this higher in-line loss.²⁵ Upper respiratory tract deposition was higher for the vibrating mesh nebulizer (17.6% vs 8.6% of the nominal dose), most of this was in the nasal cavity and nasopharynx. About 20% of the nominal dose was lost to the environment for both nebulizers. The vibrating mesh nebulizer had a higher emitted dose than jet nebulizer (97.3% vs 56.6% of the nominal dose).²⁵ That means about half of the dose was retained in the jet nebulizer and shows the higher efficiency and lower dead volume of vibrating mesh nebulizers. The lung doses of the two nebulizers were comparable if they were expressed with respect to the respective emitted doses. The dose and PI measured in each lung were similar for both nebulizers so there was no preferential deposition on either side.²⁵

The performance of nasal high-flow nebulization in pediatric patients has been evaluated in an in vivo macaque model (*Macaca fascicularis*) and an in vitro SAINT model.²⁶ The former represents a full-term newborn infant and the latter a nine-month-old toddler. An Aerogen Solo vibrating mesh nebulizer and a Cirrus2 jet nebulizer was placed upstream of the humidifier. The high flow circuit delivered fully humidified 100% oxygen at 37°C at 2, 4, and 8 L/min with the vibrating mesh nebulizer and at 8 L/min with the Cirrus2 jet nebulizer.²⁶

The jet nebulizer operated at 6 L/min so the circuit contributed 2 L/min to achieve a total flowrate of 8 L/min.

Normal saline containing [^{99m}Tc]DTPA was nebulized to both models via a neonatal-sized cannula. The macaques breathed simultaneously, whereas the SAINT model had simulated tidal breathing with a tidal volume of 25 mL with an inspiratory time of 35% of the respiratory cycle at 40 cycles per minute. The volume median diameters of the droplets produced from the vibrating mesh and jet nebulizers were 4.7 and 4.0 μm, respectively.²⁶ However, the size of the droplets that exited the nasal cannula became 1-1.4 μm for both nebulizers. Droplet size increased with flowrate for the vibrating mesh nebulizer. The reduction of droplet size through the high flow circuit was most likely due to in-line impaction of the larger droplets. Lung deposition of the aerosols in the two models is shown in Table 1. Neither nebulizer was efficient in delivering the aerosols as lung deposition was very low (< 5% for all conditions). Lung deposition decreased with increasing flowrate so the best result was with the vibrating mesh nebulizer at 2 L/min in both models (Table 1).

However, even so, lung deposition was only 0.85% and 4.15% in the macaque and SAINT models, respectively. Central-to-peripheral deposition was about one for all conditions. Extrathoracic deposition was between 3%-10% and 1.9%-12% in the macaque and SAINT models, respectively.²⁶ Increasing flowrate was associated with decreased humidifier deposition and increased cannula deposition in both models, while tubing deposition was not affected. However, the SAINT model generally had higher cannula deposition than the macaques (13.5%-46.8% vs 2.62%-28.4%). The low-lung deposition might be partly due to possible obstruction of the nasal area by the cannula. However, the most likely reason was the high flowrate of ≥2 L/min relatively to the tidal breathing rate causing gas leakage from the cannula to the environment, especially when during open-mouth breathing.²⁶ The integration of the gas flow from the jet nebulizer with that from the circuit might increase turbulence and hence in-line impaction deposition. This might explain the lower lung deposition for the jet nebulizer at 8 L/min compared to that for the vibration mesh nebulizer at the same total flowrate. The vibration mesh nebulizer did not contribute extra airflow in the line. Nevertheless, the inefficiency of nasal high flow nebulization discussed above indicates that much research is required to improve aerosol delivery through these systems.

Intranasal Deposition

Intranasal drug delivery is usually intended for local treatment. However, it can also be an alternative route of administration for reaching the central nervous system via the

Table 1 Lung Deposition from Nasal High Flow Nebulization in Macaque and SAINT Models. Adapted from Réminiac et al²⁶

	Vibrating Mesh Nebulizer			Jet Nebulizer
	2 L/min	4 L/min	8 L/min	8 L/min
Total Flowrate	2 L/min	4 L/min	8 L/min	8 L/min
Macaque Model	0.85%	0.49%	0.09%	0.03%
SAINTE Model	4.15%	3.29%	0.52%	0.46%

olfactory bulb. As for pulmonary deposition, various factors affecting intranasal deposition have been investigated in scintigraphic studies.

Laube et al.²⁷ examined the deposition of aerosols produced from Accuspray in pediatric anatomical models. Accuspray is the device for the intranasal influenza vaccine, FluMist. Nasal cavity models made from CT scans of two-, five-, and 12-year-old children were used for the experiment.²⁷ Distilled water containing TcO_4^- was sprayed into these models with the Accuspray device inserted into or placed outside the right nostril. The deposition was assessed by 2D gamma scintigraphy and quantified with respect to the emitted dose. The mean droplet size generated from Accuspray was about 68 μm , which were sufficiently large for intranasal deposition.²⁷ Most nasal products produce particles or droplets of 20–100 μm . When the Accuspray device was inserted into the nostril, deposition in the main nasal cavity was the lowest in the two-year-old (46.8%), followed by the 12-year-old (72.1%) and five-year-old (75.4%).²⁷ When placed outside the nostril, the corresponding deposition were much lower and increased with age (1.4%, 7.4%, and 21.1% for two-, five-, and 12-year-old, respectively). Variations in the intranasal regional deposition between experimental runs might be due to differences in device alignment with the nasal models.²⁷ It could also be contributed by the poor control of plunger depression by the operator, consequently leading to variations in droplet size. The higher deposition obtained when the device was inserted into the nostril could be attributed to the shorter distance that the droplets needed to traverse to the main nasal cavity.²⁷ It could also be due to less of the dose depositing outside the nose or leaked from the nostril when the device was inserted.

The type of formulation and device can affect the site of intranasal deposition through altering the size and velocity of the emitted particles, as well as the volume of the delivered dose. Deposition in the nonciliated anterior nasal cavity would have longer retention due to the lack of drug absorption and mucociliary clearance in that region. Conversely, deposition in the posterior nasal cavity would have shorter retention due to clearance from the ciliated mucosa there. This effect was shown in a comparison between the OptiNose powder device and a Rexam SP270 liquid spray pump.²⁸ The powder device and liquid spray pump generated $^{99\text{m}}\text{Tc}$ -labeled aerosols containing lactose particles (mean particle size = 15 μm) and saline (mean droplet size = 48.5 μm), respectively. Both the powder and liquid spray deposited in all nasal regions in healthy adult subjects. However, the powder deposited predominantly in the upper and middle posterior regions than the liquid spray (53.6% vs 15.7%).²⁸ On the other hand, more liquid spray deposited in the nonciliated anterior regions than the powder (15.1% vs 8.8%). Thus the powder showed more rapid overall clearance and the liquid spray had longer retention.²⁸ A similar trend was observed between a nasal solution HFA-MDI containing ciclesonide (37 $\mu\text{g}/50 \mu\text{L}$ per actuation) and an aqueous suspension nasal spray containing mometasone furoate monohydrate (50 $\mu\text{g}/100 \mu\text{L}$ per actuation).²⁹ Both were radiolabeled with $^{99\text{m}}\text{Tc}$ and administered to adult subject at a maximum radioactivity of 5 MBq per dose. The

MDI spray deposited mainly in the anterior nasal region, whereas the aqueous spray deposited more posteriorly.²⁹ Thus the aqueous spray was cleared faster overall than the MDI spray. This was reflected in the decline in radioactivity over 10 minutes postdose (32% vs 19%, respectively).²⁹ The aqueous spray also showed more loss into the nasopharynx and run-out from the nose, which could be due to its larger volume of nonvolatile liquid dispensed per actuation.

Conclusion

Gamma scintigraphy is a powerful technique for investigating particle deposition in the respiratory tract quantitatively and qualitatively. It can provide detailed information on the location and amount of deposits non-invasively thus is well suited for investigating the effects of various factors on deposition. A deeper knowledge of these factors will improve the predictability, control, and especially in vitro/in vivo correlation of aerosol deposition. Moreover, there are some physicochemical factors that have not been, but are worth to be, studied in vivo using scintigraphy. Particle electrostatic charge and surface roughness are obvious examples as their effects have already been examined in vitro. There is potential for development in these and other aspects of respiratory drug delivery with the aid of scintigraphy.

Acknowledgement

H-KC wishes to acknowledge that this research was supported under the Australian Research Council Discovery Project funding scheme (DP160102577).

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