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Regional Distribution of Aerosol Deposition in Rat Lungs Using Magnetic Resonance Imaging

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Abstract

The toxic or therapeutic effect of an inhaled aerosol is highly dependent upon the site and extent of deposition in the lung. A novel MRI-based method was used to quantify the spatial distribution of particles in the rat lung. Rats were exposed to 0.95 μm -diameter iron oxide particles in a controlled manner (N=6) or to particle-free air (N=6). Lungs were fixed in 3 % glutaraldehyde by vascular perfusion, excised and imaged in a 3T scanner using a gradient-echo imaging protocol. The signal decay rate, R_2^* , was measured in each voxel of the entire left lung (1 mm thick slices). R_2^* was significantly higher in exposed animals ($0.0065 \pm 0.0006 \text{ msec}^{-1}$) than in controls ($0.0050 \pm 0.0003 \text{ msec}^{-1}$, $p < 0.001$). A calibration curve between R_2^* and concentration of deposited particles (C_{part}) was obtained by imaging gel samples with known particle concentrations. Regional deposition was assessed by comparing C_{part} between the outer ($C_{\text{part,peripheral}}$) and inner ($C_{\text{part,central}}$) areas on each transaxial slice, and expressed as the $\frac{c}{p}$ ratio. $C_{\text{part,peripheral}}$ ($1.54 \pm 0.70 \frac{\mu\text{g}}{\text{ml}}$) was significantly higher than $C_{\text{part,central}}$ ($1.00 \pm 0.39 \frac{\mu\text{g}}{\text{ml}}$, $p < 0.05$), resulting in a $\frac{c}{p}$ ratio of 0.65. This method may be used in future studies to quantify spatial distribution of deposited particles in healthy and diseased lungs.

Keywords

particles; spatial distribution; animal model; MRI

Introduction

Inhaled aerosolized particles are widely used for therapeutic drug delivery in patients with pulmonary diseases. Selective treatment of the lungs may be achieved by delivering high drug concentrations to the airways. For example, targeted deposition may improve the efficiency of therapeutics in asthmatic patients²⁴ or lead to the ability to deliver aerosolized chemotherapy to lung cancer patients.²⁶ Additionally, the lung is increasingly being used as a portal of entry for aerosolized drugs designed to act systemically,^{12,18} e.g. insulin for diabetes patients.²⁷ The success of inhalation therapy does not only depend upon the pharmacological properties of the drugs being inhaled, but also upon the site, extent and distribution of deposition in the lung. As such, a better understanding of regional particle deposition could enhance both the safety and the efficiency of inhaled pharmaceuticals.²⁹

Several imaging methodologies have previously been developed to evaluate aerosol deposition in both human and animal subjects. These include gamma scintigraphy,^{3,5,24,28} PET,⁷ SPECT,^{8,11} and more recently MRI.^{13,19} Gamma cameras capture either 2D (gamma scintigraphy) or 3D (SPECT) images of deposited particles labeled with a radionuclide (typically ^{99m}Tc) to measure peripheral and central distribution of deposition⁵ as well as lung clearance.³ The advantages of 3D PET lie in the ability to achieve a better image quality than SPECT,⁷ as well as the capability to attach the radionuclide label directly to the drug molecule. Thus far, deposition quantification using MRI has only been performed in animal studies, using either iron oxide particles¹³ or gadolinium.^{4,9,14,19,21}

Historically rodents have been utilized to study particle deposition mainly because they are inexpensive and commercially available, laboratory rodents of the same strain are genetically identical, their anatomy is similar to humans, and highly invasive techniques may be used. Conventional slicing methods,^{16,17,22} MR imaging¹³ and CT/SPECT imaging¹¹ have been used previously to study deposition in rodents. Raabe et al.¹⁶ determined that there was an enhanced nasal-pharyngeal deposition for particles with diameter greater than $3\text{ }\mu\text{m}$, compared to particles with diameters of 1 and $0.3\text{ }\mu\text{m}$. In a different study, Raabe et al.¹⁷ found that weight-normalized particle deposition was higher in the upper right lobe (apical)² compared to the other four lobes for $1\text{ }\mu\text{m}$ -diameter particles in rats. Sweeney et al.²² found that particle deposition was lower in emphysematous hamsters, compared to healthy hamsters, for $0.45\text{ }\mu\text{m}$ -diameter particles. In mouse lungs, Martin et al.¹³ found central deposition to be higher than peripheral deposition, for particle diameters of $5.6\text{ }\mu\text{m}$. Recently, Kuehl et al.¹¹ characterized the deposition of several sizes of radioactive particles in both mice and rat lungs using 3D CT/SPECT imaging methods and found higher overall and peripheral deposition as the particle diameters were decreased from 5.0 to $0.5\text{ }\mu\text{m}$. Each of these previous studies advanced the knowledge of particle behavior in the lung. However, these studies either exposed the rodent to particles in an uncontrolled manner, used polydisperse particles,^{11,13} required slicing of the lung,^{16,22} or used imaging techniques that introduced large artifacts in the images which was caused by a large signal intensity in the vasculature.¹³

The goal of the current study was to use MRI to characterize the 3D regional distribution of deposited particles in the lungs of healthy rats. To do so, super paramagnetic iron oxide (SPIO) particles were delivered to rat lungs in a controlled manner. The set of lungs were then excised, fixed at an airway pressure of $20\text{ cm H}_2\text{O}$ and then imaged in a MR scanner. The iron particles created local disturbances in the magnetic field, which resulted in an increased signal decay rate, R_2^* , compared to particle free lung tissue. Using data analysis methods similar to those used in gamma scintigraphy,⁵ regional deposition was

characterized by the central to peripheral deposition ratio, $\frac{c}{p}$. This study is the first to quantify particle deposition in lungs using the MR signal decay rate, R_2^* . This work also improved on several previous MRI studies in animals that only reported global measures of ventilation^{4,9,14} or whole lung deposition.^{19,21}

Materials and Methods

Aerosol Delivery and Animal Preparation

The study protocol was approved by the University of California San Diego Institutional Animal Care and Use Committee (IACUC). Twelve adult male Wistar rats with mean body weight of 250 ± 14 grams were deeply anesthetized with an intraperitoneal injection of 60 mg of pentobarbital sodium per kg of body weight. Once the pain reflexes were abolished, a 15-gauge plastic catheter was inserted into the trachea.

Rats were connected to a rodent ventilator (Harvard, model # 683) and ventilated for 40

minutes at a tidal volume of 2 ml, a breathing frequency of $80 \frac{\text{breaths}}{\text{min}}$, a positive expiratory pressure (Peep) of 1 cm H_2O , and a maximum pressure of 11.2 ± 0.8 cm H_2O , measured at the trachea. Twenty minutes into the delivery, and while the rats were still connected to the ventilator, 500 units of heparin were delivered intraperitoneally to inhibit intravascular blood coagulation. Six control rats were ventilated with room air and six rats were ventilated with room air containing aerosolized SPIO particles. The particle delivery system is shown in Figure 1.

The aerosol was made of monodisperse magnetic polystyrene particles with a geometric diameter of $0.95 \mu m$ and a coefficient of variation of $< 5\%$ (Kisher Biotechnologies). The particles were made by coating a layer of iron oxide and polystyrene on polystyrene core

particles and they had a density of $1.35 \frac{g}{cm^3}$. The particles were supplied in suspension ($25 \frac{mg}{ml}$ water), and the concentrate was diluted in a 1:4 ratio with water and dispensed via an Acorn II nebulizer (Marquet Medical Products, Inc). Before being stored in a bag, the aerosol flowed through a heated tube and a diffusion dryer to remove water droplets. The aerosol generated by the nebulizer was checked with a particle sizer (APS3321, TSI) in

series with an aerosol diluter (3302A, TSI). The aerosol concentration was $\sim 5000 \frac{\text{particles}}{ml}$ of air. Size analysis showed that the fraction of doublets and triplets in the aerosol was $< 10\%$ with a mean geometric diameter of $1.04 \mu m$. When doublets and triplets were excluded from the calculation, mean geometric diameter was $0.94 \mu m$ confirming the size provided by the manufacturer. MMAD of the samples was $1.218 \mu m$.

After the aerosol exposure, rats were given a lethal dose of pentobarbital sodium intraperitoneally. The blood was removed from the lungs by vascular perfusion with a mixture of heparin and saline (1mL heparin: 100 mL saline) through the pulmonary artery at a pressure of 15 cm H_2O for 10 minutes. The lungs were then perfusion fixed at an airway pressure of 20 cm H_2O with fixative (3 percent glutaraldehyde in 0.01 M phosphate buffer

solution) for 20 minutes at a flow rate of $10 - 15 \frac{ml}{min}$ at a pressure of 15 cm H_2O . The lungs were excised and stored in custom-made MR compatible containers. Each container held three sets of lungs immersed in fixative and held in place with gauze. Prior to imaging, each container was degassed under a light vacuum for approximately two weeks until all air bubbles were removed from the lungs. The container was then placed into the middle of a larger container of water (see Field Homogeneity section). This process minimized air/tissue/container interface artifacts during MR imaging.

Imaging

All MR imaging was performed on a 3T General Electric Signa HDX MR scanner. A gradient echo sequence was used with a repetition time (TR) of 2 sec, a flip angle of 20° and a field of view of 13 cm. The polyacrylate MR compatible container was placed in the center of the GE 18 cm diameter transmit and receive knee coil. Using the smallest diameter imaging coil that accommodated the sample container, maximized available signal to noise ratio. Approximately 32 transaxial images were obtained for four echo times (TE). The four TEs were chosen to fully describe the decay of the signal intensity. The shortest TE was 8.2 msec, the minimum prescribable by the scanner for the specified set of imaging parameters. The longest TE was chosen such that the resulting signal intensity was at least three times the standard deviation of the background noise. The resulting images had an in-plane resolution of $500 \mu m$ and a thickness of 1 mm. Three scan protocols were performed: field

homogeneity validation, particle concentration calibration, and lung imaging. Each protocol required unique TEs and these are given in each respective section.

Calculation of R_2^*

The signal intensity decay for increasing echo time is characterized by the decay rate, R_2^* , where R_2^* is the reciprocal of the signal decay time constant, T_2^* . The rate of the signal decay can be approximated by

$$SI = A_0 e^{-R_2^* TE} \quad (1)$$

where SI is the signal intensity, A_0 is an unknown parameter that represents the signal intensity when TE is zero, and R_2^* is the rate of decay of the SI. An example of the decay curves are shown on Figure 2. For each transaxial slice, R_2^* was calculated for each voxel by linear regression of the log of equation 1. Only TEs for which the signal intensity was larger than three times the standard deviation of the background noise were used for the linear fits. Therefore, in some cases (less than 5 %), only three echo times were included in the linear regression. The coefficient of determination, R^2 , was calculated for each fit and only voxels with $R^2 > 0.75$ were used for the statistical analysis.

Field Homogeneity

Prior to imaging the rat lungs, a field homogeneity analysis was performed. The container was filled with fixative and imaged with TEs of 8.2, 40, 100 and 300 msec. The measured signal decay rate, R_2^* , resulted from both the fluid being imaged (R_2) and the inhomogeneities in the magnetic field (R_2'), $R_2^* = R_2 + R_2'$. The inhomogeneities in the magnetic field, R_2' , may be impacted by the RF coil, air/tissue interfaces and objects that induce magnetic susceptibility.

Large inhomogeneities were found when the polyacrylate container was filled with only fixative. Imaging was repeated with the container secured into the center of a cylinder of water with a diameter approximately 4 cm larger and 10 cm longer than the container, which served to minimize the inhomogeneities. The signal decay rate, R_2^* , was calculated for each voxel and ROIs were drawn for each transaxial slice to calculate the mean and SD of R_2^* .

Particle Concentration Calibration

A calibration curve relating R_2^* and concentration of deposited particles was obtained by imaging known particle concentrations. Two containers were filled with a gel mixture (0.75 percent agarose with fixative) and punched with six evenly spaced cylindrical holes of 5 mm in diameter. The holes were filled with known particle concentrations of 0, 1.56, 3.13, 6.25, 12.5, 25.0 $\frac{\mu g}{mL}$ suspended in the agarose gel mixture (Figure 3, left panel). The containers were imaged with TEs of 8.2, 20, 40 and 80 msec. R_2^* was calculated for each voxel. ROIs were drawn around each particle concentration mixture for ~ 12 transaxial slices. Linear regression with 95 % confidence intervals was performed to find the relationship between R_2^* and the known particle concentrations. An additional phantom experiment was performed where phosphate buffer solution (PBS) and agarose were used to create the phantom in order to test the sensitivity of the resulting slope on the medium in which the particles were embedded.

Lung Particle Deposition

Each container of three sets of rat lungs were imaged at TEs of 8.2, 40, 100 and 200 msec, with an imaging time of approximately 3.5 hours. Imaging time was determined by several factors including 1) the repetition time (TR = 2 sec), so that longitudinal effects on the MR signal could be minimized, 2) the number of echo times (four in this sequence), 3) the number of phase encoding steps (i.e. 256 steps), 4) the number of slices (~32), and 5) the fact that data was collected in duplicate, to reduce the signal to noise ratio. Mean and standard deviation of R_2^* values were computed from each slice by drawing ROIs around the left lung. ROIs were defined for each rat individually and the main bronchioles were used as an anatomical reference. The left lung was selected for analysis because it comprises of only one lobe, whereas the right lung consists of four lobes. Sub ROIs delimiting the central and peripheral regions were defined as follows: for the transaxial slices located in the mid-portion of the lung (i.e. for the slices encompassing the region surrounding the main bronchus), both a central and peripheral region was defined on each slice as shown in Figure 4, while the transaxial slices located at the apex and the base of the lung only included peripheral tissue. The central region was then summed together to form a 3D central region. Similarly, all peripheral regions were added together to form a 3D peripheral region. In this manner the central region made up about 25% of the lung with the remaining 75% consisted of the peripheral region. With this definition, both the central and peripheral regions contained small airways and parenchyma, whereas the central region also included large and

medium sized airways. Relative dispersion ($RD = \frac{SD}{mean}$) was computed for each ROI. The higher the RD, the more heterogeneous the R_2^* measurement within the ROI.

On average, the left lung spanned 23 ± 3 slices of the 32 images within the MR data set. The distribution of R_2^* along the apex to base of the left lung was assessed by grouping slices in ten bins uniformly distributed along the apex-base axis. Each bin contained R_2^* values for two or three images (ie: covering approximately 3 mm in the superior inferior direction).

Particle concentration (C_{part}) was computed by

$$C_{part} = \frac{R_2^* - R_{2,control}^*}{m} \quad (2)$$

where $R_{2,control}^*$ is the mean R_2^* for all of the control lungs and m is the slope calculated from the particle concentration calibration experiment (Figure 3). The implicit assumption made by using the same slope (m) to calculate the concentration of deposited particles in the lung is that the presence of particles affects R_2^* independently of the medium in which the particles were embedded in (lung tissue in fixative or agarose-fixative solution). A similar assumption was made in a previous particle concentration MR study by Martin et al.¹³ who used a linear relationship between particle concentration and T_1 measurements to quantify particle deposition in mice.

Particle concentration was calculated for each voxel within each ROI for all control and exposed lungs. For each rat, normalized regional C_{part} was obtained by dividing each voxel in the central and peripheral regions by the mean C_{part} for the entire left lung. Mean and SD

of central and peripheral particle concentration ($\frac{\mu g}{mL}$) was computed from all the voxels spanning each 3D region for each exposed rat. the mean particle concentration of the central

(c) and peripheral (p) regions was used to calculate the central to peripheral ratio, $\frac{c}{p}$.

Finally, to check for gravitational dependence on particle deposition resulting from any gravitationally-induced differences in ventilation, two additional ROIs were drawn. The ROIs were defined such that approximately half of the lung was outlined as the dependent (dorsal) and half the lung was the non-dependent (ventral) regions of the exposed lung. The ratio of dependent to non-dependent region of the lung was then calculated.

Statistical Analysis

Data for R_2^* , RD and C_{part} were grouped on the basis of three categorical variables: (i) percentaxial distance from the apex of the lung (10 levels), (ii) ROI (2 levels: central and peripheral), and (iii) exposure condition (2 levels: control and exposed lungs). A two-way ANOVA for correlated samples was used to test for differences in R_2^* across the lung depth for the control and exposed lungs (Figure 6), and to test for differences in R_2^* , RD, and C_{part} for each ROI for the control and exposed lungs (Table 1). Post-ANOVA, pair-wise comparisons using Bonferroni adjustments were performed for tests showing significant F ratios. To compare each region within each category (i.e.: control and exposed separately) a one-way ANOVA test was used with the Tukey Multiple Comparison post-hoc test (Table 1). The paired t-test was used to compare overall central to peripheral concentrations. Significant differences were accepted at the $p < 0.05$ level. Outliers were determined using the Grubbs test and removed from the data analyses. All statistical analyses were performed with Prism (GraphPad, San Diego, CA). One set of lungs from the exposed group was removed from the data shown in Figure 6 as it was determined to be a statistical outlier and its R_2^* values were similar to the control lungs, suggesting that this rat did not receive any particles.

Results

Field Homogeneity

Figure 5 shows the R_2^* averaged in the axial direction and the respective SD within slices for the field homogeneity tests. When the polyacrylate container was imaged on its own, R_2^* varied considerably both between slices and within an image slice, as demonstrated by the large SD bars. The signal decay rate was greatest at both ends of the container and least in the center slices. In contrast, when the container was submerged in a larger cylinder filled with water, R_2^* did not vary either in the axial direction or within each slice. These results suggest that the field inhomogeneities in the container arise predominately from the air/container interface and these field inhomogeneities were removed when the polyacrylate container was submerged in the large cylinder of water. Therefore, for all subsequent imaging sessions the container was submerged and secured in the center of the water-filled cylinder.

Particle Concentration Calibration

The left panel of Figure 3 shows the R_2^* map of the particle concentration phantom. Particle concentration was highest for the brightest circle and decreases counterclockwise around the image. The right panel of Figure 3 shows the measured R_2^* for the two experiments performed with the phantom made of a solution of agarose in fixative and the linear relationship between the measured R_2^* and the known particle concentration. The slope of the calibration curve was $0.00107 \frac{\mu\text{g}}{\text{mL} \cdot \text{sec}}$ with a R^2 of 0.98. The slope was re-calculated using only the data for the three lowest particle concentrations (0, 1.56, $3.13 \frac{\mu\text{g}}{\text{mL}}$) and was $0.00105 \frac{\mu\text{g}}{\text{mL} \cdot \text{sec}}$. This small change (1.7 %) suggests that the particle slope was not greatly

influenced by the higher concentrations. The slope of the calibration curve from the phantom made with PBS was $0.00126 \frac{\mu\text{g}}{\text{mL} \cdot \text{msec}}$ and was not statistically different from that obtained with the fixative-agarose phantom ($p = 0.876$).

R_2^* in the Control and Exposed Lungs

An example of the increased signal decay rate in a voxel containing particles and lung tissue, compared to a particle-free voxel with only lung tissue, is shown in Figure 2. One set of lungs from the exposed group was removed from the data shown in Figure 6, as it was determined to be a statistical outlier, and the R_2^* values were similar to the control lungs, suggesting that there likely was an error in the particle exposure for this rat. The R_2^* values are plotted in Figure 6 based on their apex to base location in the lung for both the control (open squares) and exposed lungs (black circles). Data are shown as means $\pm$ SD, averaged over all animals in each group. Overall, R_2^* in the exposed lungs was significantly higher than in the control lungs ($p < 0.001$) for the entire left lung. In each group, R_2^* was not affected by the slice's superior-inferior position, except at the base and apex of the lung of the exposed animals, where R_2^* was significantly higher than in the other slices (see Figure 6). As there was little variance in R_2^* in the superior-inferior direction of the lung, the bin averaged R_2^* did not depend on the number of image slices within a bin. R_2^* and its relative dispersion are listed in Table 1 for the entire left lung and for the central and peripheral regions, respectively. Statistical comparison between the control and exposed groups is also shown along with the statistical analysis within each group. The relative regional volume of the central and peripheral regions were $26.6 \pm 1.6 \%$ and $73.3 \pm 2.3 \%$ for the control lungs and $25.8 \pm 3.9 \%$ and $74.1 \pm 4.4 \%$ for the exposed lungs, respectively. For all regions, R_2^* was significantly higher in the exposed lungs than in the control lung. As expected, there was no significant difference in R_2^* between regions for the control lungs. In the exposed lungs, R_2^* was significantly lower in the central region when compared to the entire lung and was significantly higher in the peripheral region compared to the central region. However, R_2^* in the peripheral region did not significantly differ from R_2^* in the entire lung.

Compared to controls, RD was significantly higher for all regions of the exposed lungs (Table 1). Within each group, there was a significantly higher RD in the central region when compared to the entire lung with a relative increase of $27.8 \pm 20.0 \%$ and $17.9 \pm 13.2 \%$ for the control and exposed lungs, respectively.

Particle Concentration

The particle concentration in the exposed lung was $1.42 \pm 0.60 \frac{\mu\text{g}}{\text{mL}}$ (mean $\pm$ SD) with a 95 % confidence interval ranging from 1.34 to $1.49 \frac{\mu\text{g}}{\text{mL}}$. Using the slope derived from the PBS phantom, the particle concentration was $1.19 \frac{\mu\text{g}}{\text{mL}}$, i.e: 16 % lower than the particle concentration calculated from the calibration curve obtained from the phantom made with fixative. The central to peripheral ratio, $\frac{c}{p}$, was 0.65 ± 0.12 . The $\frac{c}{p}$ ratio was independent of the slope used, as the relationship between R_2^* and particle concentration was linear. To eliminate any differences in exposure concentrations, the regional particle concentration was normalized by the total particle concentration for each rat. The peripheral normalized concentration (1.10 ± 0.04) was significantly higher ($p < 0.003$) than the central normalized concentration (0.68 ± 0.11). This normalized particle concentration was similar between rats, with a RD of 0.15 and 0.03 for the central and peripheral regions, respectively,

However, there was a high variability in particle concentration within each rat, for both the central ($RD = 2.45 \pm 1.53$) and peripheral regions ($RD = 1.18 \pm 0.51$).

In the gravitational analysis, the ratio of the concentration of deposited particles in the non-dependent to the dependent region of the lung was 1.45 ± 0.77 . This gravitational ratio was not statistically different than 1, suggesting that there was no relationship between particle deposition and gravity.

Discussion

The principal objective of this study was to use magnetic resonance imaging to determine the regional distribution of aerosol deposition in the lungs of small animals. Few studies have used aerosolized MR contrast agents to evaluate pulmonary drug delivery^{13,19} and the majority of these previous studies have used MR contrast agents to study regional lung ventilation.^{4,9,14,21} Sood et al.¹⁹ ventilated piglets with aerosolized Gd-DTPA and imaged them in a 4T MR scanner using a T_1 weighted spin-echo sequence. Pulmonary aerosol deposition was then assessed by increases in signal intensity on the MR images of exposed animals compared to baseline conditions in the same animals. Martin et al.¹³ exposed mice to iron oxide particles and used an inversion-recovery fast spin echo pulse sequence to quantify longitudinal relaxation time (T_1) of the lungs. Regional concentration of deposited particles was then assessed by differences in T_1 values between a group of exposed mice and a group of controls. In this study, we focused on the decay rate, R_2^* , to quantify aerosol deposition in the lungs of ventilated Wistar rats. We showed that lungs exposed to SPIO particles had a significantly higher R_2^* than particle-free lungs. Additionally, we found that for these particles, the deposition in the peripheral region was significantly higher than in the

central region, with a $\frac{c}{p}$ of 0.65 ± 0.12 .

R_2^* in the Control and Exposed Lungs

R_2^* was significantly higher in the exposed lungs (Figure 6) because of the increase in magnetic field inhomogeneities resulting from the presence of SPIO particles in the lung tissues. While R_2^* was also relatively constant over most of the lung slices of the exposed animals, R_2^* was higher at the base and apex of the lungs when compared to the other slices (Figure 6). While the central slices contained both lung parenchyma (peripheral region) and conducting airways (central region), the base and the apex of the lung mainly included lung parenchyma for which R_2^* was significantly higher than in the central region (Table 1).

Therefore, the higher R_2^* at the apex and base of the lung is likely the result of these regions containing only peripheral tissue where concentration of the deposited particles was higher than in the central region. Other factors such as variability in the lung thickness or in the MR measurements are unlikely to explain the higher R_2^* measured at the apex and the base of the lung. Indeed, variability in lung thickness from apex to base of the lung had little effect on R_2^* , as there was no change in R_2^* from the apex to the base of the control lungs. Measurement variability also appeared to be minimal based on data obtained from two different imaging sessions of the same exposed lung, where averaged R_2^* varied by about 0.08 %.

The impact of lung structure on the relative dispersion (RD) of R_2^* was characterized by RD values measured in the control lungs. The increase in RD between the control and exposed lungs reflected the dispersion which resulted from the presence of particles that were deposited in the lungs of exposed animals. RD was highest in the central region in both the control and exposed lungs (Table 1). This is likely the result of more heterogeneity in lung

density in this region as some of the large airways had diameters comparable or even larger than the in-plane voxel size of 0.5 mm. As a result, some of the voxels of the central region only contained fixative (low R_2^*) while other contained a combination of airway wall, lung parenchyma, deposited particles and fixative (high R_2^*). Comparing the central region to the entire lung, there was a higher percent change in RD for the exposed lungs compared to the control lungs, indicating that some particles deposited on the large airways. There was no difference in the percent change in RD from peripheral to entire lung region between the control and exposed lungs. However, R_2^* was higher in the peripheral region of the exposed lungs than in the control lungs. This suggests that deposition in the lung periphery was uniform between voxels, however it is unknown if this uniformity still exists at the sub-voxel level. It should also be noted that the increase in RD between control and exposed lungs most likely reflects the presence of deposited particles and is not the result of signal noise. When up to five times the standard deviation of the noise was added to the signal intensity of one exposed lung, RD increased by 3.4 % above that calculated from the original dataset (no added noise). Such an increase is much smaller than the increase in RD seen between the exposed and control rats.

Particle Concentration

Using the linear relationship found between R_2^* and particle concentration in the phantom experiment (Figure 3), the concentration of particles was determined in both the control and exposed lungs (see Table 1). As expected, particle concentration (C_{part}) averaged zero in the control lungs. The standard deviation of C_{part} in the control rats (Table 1) represents the variability in the measurements due to the presence of residual blood and tissue density unevenness, both of which affect R_2^* . However, this variability was small compared to the concentration of particles found in the exposed rats (Table 1). The uncertainty in the slope derived from the phantom calibration had a minimal impact on the particle concentration in the exposed lungs as the 95 % confidence interval of C_{part} was less than the standard deviation of C_{part} between rats. The variability in C_{part} between rats was most likely due to differences in exposure concentrations, as the variability in morphometry between rats of the same strain, weight, sex and health is minimal.¹⁵

The concentration of particles in the periphery was 54 % higher than in the central region of the left lung. These results agree with a previous study by Raabe et al.,¹⁶ where Fisher rats were exposed to radiolabeled particles ranging between 0.3 and 10 μm through nose-only breathing. Deposition in the bronchial and pulmonary regions was quantified based on estimation of total inhaled activity and on activity measured from the deposited particles in the two regions of the lung. For 1 μm -diameter particles the study found that more particles

deposited in the lung periphery than in the bronchioles. However, a $\frac{c}{p}$ ratio could not be calculated from this data as the bronchial and peripheral regions were defined differently. More recently, Kuehl et al.¹¹ investigated aerosol deposition patterns of radiolabeled polydisperse particles with MMAD of 0.5, 1, 3 and 5 μm using SPECT/CT techniques both in rats and mice. Regional deposition was quantified using an in-house Onion Model.¹¹ Similar to this study, Kuehl et al.¹¹ found that, for 1 μm particles, deposition was higher in the lung periphery than in the central airways, however a central to peripheral ratio was not provided.

While the actual number of particles was not measured during each animal exposure, deposition efficiency was estimated based on average concentration of aerosol generated by the nebulizer ($5000 \frac{\text{particles}}{\text{ml of air}}$, see Methods section) and on the calculated concentration

of deposited particles in the lung. For a breathing frequency of $80 \frac{\text{breaths}}{\text{min}}$, a tidal volume of 2 mL and an exposure time of 40 min, a total of $32 * 10^6$ particles were delivered to the rat. Assuming that aerosol was delivered to each lung in proportion to their volume (left lung is 36 % of total lung volume¹⁵), a total of $11.5 * 10^6$ particles were delivered to the left lung. Using the average concentration of deposited particles measured in this study, $2.67 * 10^6$ particles deposited in the left lung, resulting in 23 % deposition efficiency. If one assumes similar deposition efficiencies for both the right and left lung,¹ this number is also representative of deposition in the lung as a whole. Raabe et al.¹⁶ reported particle deposition as a percentage of total deposition in the pulmonary (11 %), bronchial (8.2 %), larynx (1.3 %), trachea (0.14 %), skull (1.1 %) and GI tract (7.6 %) for $1.03 \mu\text{m}$ diameter particles. Using this data, the estimated deposition percentage for particles only delivered to the lung (as is the case for mechanical ventilation) would be $\frac{19.3}{90} = 21\%$, a value close to that found in the present study.

In a modeling study, Darquenne et al.⁶ showed that the delivery of inhaled aerosols into the lung segments was dominated by convective processes for specific particle sizes and breathing flow rates characterized by the Stokes' number. The Stokes' number is a non-dimensional parameter that denotes the importance of inertial forces acting on the particle and is defined as:

$$Stk = \frac{\rho_p d_p^2 u}{18 \mu d} \quad (3)$$

where ρ_p is the density of the particle, d_p is the diameter of the particle, u is the velocity of the fluid, μ is the viscosity of air and d is the diameter of the airway. The study of Darquenne et al.⁶ suggested that when the Stokes' number within the trachea was less than 0.01, particles and gas flow were proportionally distributed to the lung segments with minimal deposition in the central airways. For a tracheal diameter of 0.23 cm ¹⁵ and the experimental conditions used in this study, the Stokes number in the trachea was 0.0068, supporting the findings of enhanced particle deposition in the lung periphery compared to the central region.

Finally, no differences were found in particle deposition in the gravitational dependent and non-dependent regions of the lung. The lack of gravitational dependence is most likely because the gravitational height of the lung was small. Verbanck et al.²⁵ also showed there was no gravitational influence on ventilation in the rat lung.

Comparison with other MR Techniques: Advantages and Limitations

Most of the previous MR studies of aerosol inhalation in small animals were based on the longitudinal relaxation time T_1 .^{13,19,20} Using ventilated newborn pigs, Sood et al.¹⁹ acquired serial T_1 -weighted images of the lungs, kidneys, liver, skeletal muscle and heart before and during a 90-minute exposure to aerosolized Gd-DTPA. All images were taken at the same anatomical location and showed a significant increase in signal intensity in the lungs and kidneys during aerosolization. The significant increase in signal intensity in the kidneys but not in the other organs suggested extensive alveolar absorption in the interstitial compartment, i.e the extravascular and extracellular water spaces of the lungs. They also observed higher increase in signal intensity in the kidneys compared to the lungs, which may have been due to a combination of rapid clearance from the lungs, prompt elimination and concentration by the kidneys and better MR visualization of a solid organ free of air/tissues interfaces. While images were acquired in-vivo, no quantification of aerosol deposition or

assessment of regional distribution of deposited particles was performed. In a followup study, the same group²⁰ acquired 3D images of piglets ventilated with aerosolized Gd-DTPA, however aerosol delivery to the lungs was indirectly measured through increase in signal intensity in the kidneys and again no quantification of aerosol deposition was reported.

Aerosolized iron oxide particles have also been previously used as a contrast agent to measure aerosol delivery to the lungs. Martin et al.¹³ determined the concentration of deposited particles delivered to mice in nose-only inhalation chambers. Imaging was performed post-mortem. They used a linear calibration of the changes of T_1 values to the concentration of iron particles based on the relaxation time constant of the tissue a particle calibration constant determined based on experiments in agar phantoms over the same

concentration range as in the present study (i.e. 0 to $20 \frac{\mu g}{mL}$). While performed in-situ, the lung vasculature had a large effect on T_1 values. This effect was minimized by eliminating all voxels in the lung ROI that had a signal intensity greater than a threshold value. Such post-processing resulted in the removal of a significant number of voxels mainly in the central region of the lungs. Further, the average T_1 relaxation times in control animals decreased with time after death, most likely because of redistribution of water between the airway surface liquid, interstitial, and vascular spaces of the lung. This required a correction to be applied to the data so that any decrease in T_1 was directly correlated to the presence of deposited particles. Even though it had some limitations, this study was the first attempt at quantifying the number of deposited particles in lung tissues using MRI.

The present study focuses on R_2^* decay rates rather than T_1 relaxation times to quantify distribution of deposited particles within the lungs. It also has the advantage of avoiding any effect of the vasculature on the measured signals. Indeed, blood (that also contains iron) was removed from the lung vasculature before the lung was perfusion-fixed at a controlled lung volume. Lungs were fixed through the vasculature rather than by instillation in order to minimize any potential dislocation of deposited particles during the fixation process. Once fixed, the lungs were submerged in fixative under gentle vacuum to remove air from the lungs. Doing so minimized potential air/tissue artifacts that could alter the measurements. Unlike in the Martin et al. study,¹³ all voxels in the selected ROI could be used, as there was no interference between the signal from the vasculature and from the presence of iron particles. As such, regional distribution of deposited particles could be performed for various ROIs. However, the technique did not allow for absolute quantification of lung deposition as no in-line monitoring of inhaled concentration was available in the exposure system. Finally, it should be noted that both the technique presented here and that used by

Martin et al.¹³ allow for detecting similar levels of particle concentration ($\sim 1 \frac{\mu g}{mL}$).

While the resolution ($0.5 \times 0.5 \times 1$ mm) of the MR data presented here was sufficient to determine regional deposition, individual airways were not clearly distinguishable on the images except for the large airways. An increased resolution may allow for detecting particles deposited on medium-sized airways. Such higher resolution may be obtained in MR scanner with stronger magnetic field (i.e.: 7T or 11.7T) than that used in this study (3T) without sacrificing signal intensity. However, the larger magnetic field may cause an increase in air-tissue interface signal decay and therefore making the imaging more susceptible to trapped air bubbles, blood and other causes of susceptibility artifacts.

While all these studies performed in animals with Gd-DTPA or iron particles showed the feasibility of using MRI to measure aerosol delivery, only one preliminary study¹⁰ has been performed in humans to date. To apply these MR techniques to human studies, one has to

consider the sensitivity of the techniques, i.e. their ability to detect changes in relaxation times induced by the presence of particles. Based on studies in animals, Thompson and Finlay²³ estimated that using Gd-DTPA, a relatively large amount of contrast agent (~ 0.5 mg) per voxel would be needed while much smaller amount of iron oxide would be required. Finally, it should be noted that while techniques used by Sood et al²⁰ and Martin et al¹³ have the potential to be applied to humans, because of its invasiveness, the technique described in this paper is not intended for use in humans but rather in animals where detailed maps of deposited particles can be obtained both in health and disease models.

Comparison with Radionuclide Imaging Techniques: Advantages and Limitations

Other imaging methods, such as planar gamma scintigraphy,^{3,5,24,28} PET⁷ and SPECT,^{8,11} are also used to study aerosol deposition and clearance. Planar gamma scintigraphy and SPECT typically image ^{99m}Tc radiolabeled particles, whereas PET uses positron-emitting radionuclides that may be attached directly to the drug molecule. Planar gamma scintigraphy is the most widely used technique, but data can only be viewed in two dimensions, which limits regional deposition analysis. Also, the 2D nature of the images complicates the analysis of the left lung because of overlapping activity from the stomach and scattering from nearby tissues. These limitations disappear with SPECT, as it provides 3D images of aerosol deposition. However, analysis of deposition data is more complex for SPECT than for data obtained by planar gamma scintigraphy mainly because of challenges in alignment of data from the two cameras and possible corrections needed due to scan times being longer than absorption times of the radionuclide. PET provides greater image resolution than gamma scintigraphy or SPECT, however PET scanners are currently not widely available. Furthermore, attaching the radionuclides to the drug molecule is expensive and half-lives of the radiotracers are rather short. In order to relate the deposition maps to anatomy, all three imaging modalities require additional CT imaging.

Several studies have used radionuclide imaging to study aerosol deposition in healthy^{1,11} and diseased rodents.²² Using scintillation counting on small lung sections, Asgharian et al.¹ measured deposition in rats following nose-only exposure to ⁵⁶FeCl₃ particles with aerodynamic diameters ranging from 0.9 to 4.2 μ m. They found deposition patterns to be similar among the various lobes for all particle sizes and found a maximum in total deposition for 3.4 μ m particles. Sweeney et al.²² exposed healthy and elastase-induced emphysematous hamsters to ^{99m}Tc-sulfur colloid aerosol with a MMAD of 0.45 μ m and found that total particle deposition was lower and heterogeneity of particle deposition was higher in elastase-treated animals than in controls. Additionally, they found that deposition was higher in the large airways and lower in the lung parenchymal in the elastase-treated hamsters, compared to healthy controls. Kuehl et al.¹¹ used SPECT/CT imaging to determine regional deposition in healthy rats and mice exposed to radiolabeled polydispersed particles with MMAD of 0.5, 1.0 3.0 and 5.0 μ m. They showed that particle deposition in the lung increased with decreasing particle size, whereas the deposition in the oral/nasal area increased with increasing particle size. Regional deposition analysis using an onion-skin type model also showed increasing peripheral deposition with decreasing particles size.

To our knowledge, Kuehl et al's¹¹ study is the only study currently available in the literature that used SPECT to assess regional deposition in rodent lungs. This study is also probably the most comparable to our MRI dataset. As similar image resolution was used in both studies ($0.4 \times 0.4 \times 0.4$ mm¹¹ compared to $0.5 \times 0.5 \times 1.0$ mm in this current study), no resolution advantage is shown with either SPECT or MRI in small animal imaging. However, compared to the current study, advantages in SPECT lay 1) in the ability to image the lungs in-situ therefore requiring less sample preparation and 2) in the ability to quantify

particle burden in other organs such as stomach and liver. As with the MRI method presented here, the deposition quantified by Kuehl et al.¹¹ needs to be validated.

Conclusion

This study demonstrated the feasibility of using MRI to detect and quantify regional aerosol particle deposition in the lung. While previous MR studies^{13,19} have utilized the reduction in T_1 relaxation times to quantify regional delivery of inhaled aerosol, this study evaluated the potential use of R_2^* measurements to assess aerosol deposition, as R_2^* is sensitive to the local field inhomogeneities caused by the SPIO particles. We showed that, compared to control animals, R_2^* was higher in rats exposed to $0.95\ \mu\text{m}$ particles and that R_2^* was proportional to the concentration of deposited particles. In agreement with previous studies,^{11,16} we also showed that particle deposition was higher in the lung periphery than in the central region as

evidenced by a $\frac{c}{p}$ ratio of 0.65. These data strongly support the use of MRI techniques to assess aerosol deposition in small animals by measuring signal decay rate R_2^* . The methods described in this paper can readily be applied to various exposures with different aerosol sizes and/or in animal with various disease models.

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Figure 1.

Rat aerosol delivery experiment configuration. Aerosol was generated with a nebulizer, dried by going through a heated diffusion dryer and then stored in a rubber aerosol bag. The mechanical ventilator pushed the aerosol into the rat lung during inhalation and then the rat passively exhaled into the P_{peep} jar during exhalation.

Figure 2.

Example of the decay of the signal intensity for increasing TE for a voxel from a particle-free and exposed lung, respectively. In this example, signal intensity was the same for both voxels at the echo time of 100 msec. Exponential fits were found by linear regression of the log of the signal intensity. The R_2^* is higher (signal decay rate is more rapid) in the voxel containing the SPIO particles.

Figure 3.

Left Panel: R_2^* map derived from MR images of the particle concentration phantom with known particle concentrations. Brightest circle has the highest particle concentration and darkest circle (non detectable on this image) does not contain any particles. Right Panel: The linear relationship between R_2^* and the particle concentration. Data are shown as mean with error bars corresponding to the inter-voxel variability (SD) of particle concentration.

Figure 4.

Top Panel: An example MR image of the polyacrylate container housing three sets of rat lungs. A control lung is shown in the top left position and two exposed lungs in the bottom and right positions of the container. The lung outlined in blue is an example of an ROI drawn for the left lung. Bottom Panel: The two regions analyzed; the outer (purple) ROI represents the periphery of the lung and the inner (green) ROI represents the central region, which contains large and small airways as well as peripheral tissue.

Figure 5.

R_2^* measurements from field homogeneity experiments. The circles represent the measurements when the polyacrylate container was imaged on its own and the squares represent data from the polyacrylate container submerged in a larger container filled with water, with error bars representing the inter-voxel variation (SD) within an image (SD for the data with the submerged container is smaller than the symbols). The submerged container showed no R_2^* variation along the axial axis as well as a small standard deviation within the image.

Figure 6.

Average R_2^* measurements from the apex to the base of the left lung for the control and exposed rats. Error bars represents the inter-rat variability (SD). The R_2^* measurements did not change significantly in the axial direction for the control regions. The base R_2^* was significantly different ($p < 0.05$) from slices marked with x and the apex R_2^* was significantly ($p < 0.05$) different from slices marked with \$. * $p < 0.001$

R_2^* Relative Dispersion (RD) and Particle Concentration for the Entire, Central and Peripheral regions of the left lung with statistical comparison between group (E vs. C) and within each group (C, E). $\uparrow$ ($\downarrow$) indicate an increase (decrease) for $p < 0.05$, $p < 0.01$, $p < 0.001$, with 1, 2 or 3 arrows, respectively.

Table 1

$R_2^* msec^{-1}$					
	Control (C)	Exposed (E)	E vs. C	C	E
Entire Lung	0.0050 ± 0.0003	0.0065 ± 0.0006	$\uparrow \uparrow \uparrow$	Central vs. Entire N.S.	$\downarrow$
Central	0.0051 ± 0.0003	0.0061 ± 0.0004	$\uparrow \uparrow$	Peripheral vs. Entire N.S.	N.S.
Peripheral	0.0050 ± 0.0003	0.0067 ± 0.0004	$\uparrow \uparrow \uparrow$	Peripheral vs. Central N.S.	$\uparrow \uparrow$
RD of R_2^*					
	Control	Exposed	E vs. C	C	E
Entire Lung	0.192 ± 0.032	0.258 ± 0.026	$\uparrow$	Central vs. Entire $\uparrow$	$\uparrow$
Central	0.240 ± 0.049	0.309 ± 0.043	$\uparrow$	Peripheral vs. Entire N.S.	N.S.
Peripheral	0.169 ± 0.030	0.234 ± 0.025	$\uparrow$	Peripheral vs. Central $\downarrow \downarrow \downarrow$	$\downarrow \downarrow$
Particle Concentration $\frac{\mu g}{mL}$					
	Control	Exposed	E vs. C	C	E
Entire Lung	0.00 ± 0.30	1.42 ± 0.60	N.A.	Central vs. Entire N.A.	$\downarrow$
Central	0.03 ± 0.31	1.00 ± 0.39	N.A.	Peripheral vs. Entire N.A.	N.S.
Peripheral	-0.02 ± 0.30	1.54 ± 0.70	N.A.	Peripheral vs. Central N.A.	$\uparrow \uparrow$