The relative deposition index: A novel quantitative method to analyse the distributions of nanoparticles within tissues and cells - Theory and Practice

Christian Mühlfeld[1], Terry M. Mayhew[2], Marianne Geiser[1], Nadine Kapp[1], Peter Gehr[1], Barbara Rothen-Rutishauser[1]

1: University of Bern, Institute of Anatomy, Bern, Switzerland
2: University of Nottingham, School of Biomedical Sciences, Nottingham, U.K.

Summary

Background: The penetration, translocation and distribution of ultrafine and nanoparticles in tissues and cells are challenging issues particle research. Especially, translocation of nanoparticles (NP) from the pulmonary airways into other pulmonary compartments or the systemic circulation is controversially discussed in the literature. In a previous study it was shown that titanium dioxide NP were “distributed in four lung compartments (air-filled spaces, epithelium/endothelium, connective tissue, capillary lumen) in correlation with compartment size”. It was concluded that particles can move freely between these tissue compartments. To analyze whether the distribution of titanium dioxide NP in the lungs is really random or shows a preferential targeting we first established a new method for comparing the distributions of NP within tissues or cells and applied this method to the distribution of titanium dioxide NP in the lung.

Theoretical foundation: The novel quantitative microscopic methods for evaluating particle distributions within sectional images of tissues and cells address the following questions: 1) Is the observed distribution of particles between spatial compartments random?, 2) Which compartments are preferentially targeted by particles? Each of these questions can be addressed by testing an appropriate null hypothesis.

The methods require observed particle distributions to be estimated by counting the number of particles associated with each defined compartment. For studying preferential labelling of compartments, the size of each of the compartments must also be estimated by counting the number of points of a randomly-superimposed test grid which hit the different compartments. The latter provides information about the particle distribution that would be expected if the particles were randomly distributed, i.e. the expected number of particles.

From these data, we calculate a relative deposition index (RDI) by dividing the observed number of particles by the expected number of particles. The RDI indicates whether the

observed number of particles corresponds to that predicted solely by compartment size (for which RDI = 1). Within one group, the observed and expected particle distributions are compared by chi-squared analysis. The total chi-squared value indicates whether an observed distribution is random. If not, the partial chi-squared values help to identify those compartments that are preferential targets of the particles (RDI>1).

Practical application: Rat lungs exposed to an aerosol containing titanium dioxide NP were prepared for light and electron microscopy at 1h and at 24h after exposure. Numbers of titanium dioxide NP associated with each compartment were counted using energy filtering transmission electron microscopy. Compartment size was estimated by unbiased stereology from systematically sampled light micrographs. Numbers of particles were related to compartment size using the relative deposition index and chi-squared analysis.

Nanoparticle distribution within the four compartments was not random at 1h or at 24h after exposure. At 1h the connective tissue was the preferential target of the particles. At 24h the NP were preferentially located in the capillary lumen.

We conclude that titanium dioxide NP cannot move freely between pulmonary tissue compartments. The present study suggests a rapid transport from the airways to the connective tissue and a subsequent translocation to the systemic circulation.

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Background

The penetration, translocation and distribution of ultrafine and nanoparticles in tissues and cells are challenging issues in particle research[1]. Especially, translocation of nanoparticles (NP) from the pulmonary airways into other pulmonary compartments or the systemic circulation is controversially discussed in the literature. In a previous study it was shown that titanium dioxide NP were "distributed in four lung compartments (air-filled spaces, epithelium/endothelium, connective tissue, capillary lumen) in correlation with compartment size". It was concluded that particles can move freely between these tissue compartments[2]. To analyze whether the distribution of titanium dioxide NP in the lungs is really random or shows a preferential targeting we first established a new method for comparing the distributions of NP within tissues or cells and applied this method to the distribution of titanium dioxide NP in the lung.

Theoretical foundation

The novel quantitative microscopic methods for evaluating particle distributions within sectional images of tissues and cells address the following questions: 1) Is the observed distribution of particles between spatial compartments random?, 2) Which compartments are preferentially targeted by particles? Each of these questions can be addressed by testing an appropriate null hypothesis. The methods require observed particle distributions to be estimated by counting the number of particles associated with each defined compartment. For studying preferential labelling of compartments, the size of each of the compartments must also be estimated by counting the number of points of a randomly-superimposed test grid which hit the different compartments. The latter provides information about the particle distribution that would be expected if the particles were randomly distributed, i.e. the expected number of particles.From these data, we calculate a relative deposition index (RDI) by dividing the observed number of particles by the expected number of particles. The RDI indicates whether the observed number of particles corresponds to that predicted solely by compartment size (for which RDI = 1). Within one group, the observed and expected particle distributions are compared by chi-squared analysis. The total chi-squared value indicates whether an observed distribution is random. If not, the partial chi-squared values help to identify those compartments that are preferential targets of the particles (RDI>1)[3].

Practical application

Rat lungs exposed to an aerosol containing titanium dioxide NP were prepared for light and electron microscopy at 1h and at 24h after exposure. Numbers of titanium dioxide NP associated with each compartment were counted using energy filtering transmission electron microscopy. Compartment size was estimated by unbiased stereology from systematically sampled light micrographs. Numbers of particles were related to compartment size using the relative deposition index and chi-squared analysis. Nanoparticle distribution within the four compartments was not random at 1h or at 24h after exposure. At 1h the connective tissue was the preferential target of the particles. At 24h the NP were preferentially located in the capillary lumen. We conclude that titanium dioxide NP cannot move freely between pulmonary tissue compartments. The present study suggests a rapid transport from the airways to the connective tissue and a subsequent translocation to the systemic circulation[4].

Hypothetical examples

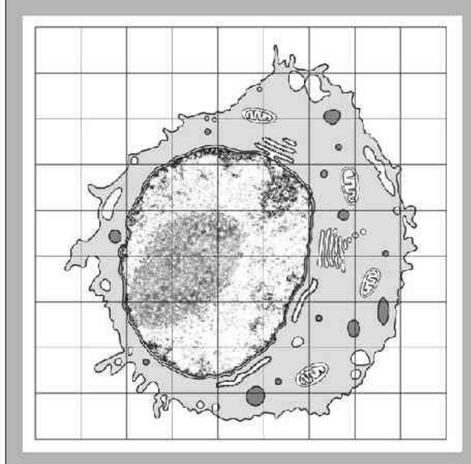

Figure 1.The use of test points to determine the volume fraction of each defined compartment in relation to cell volume and, hence, the expected number of particles. A cell profile, taken from a larger set of images, all gathered by systematic uniform random sampling, is subjected to point counting. The intersections of the lattice are taken as points. The number of points hitting each defined compartment is counted (observed points). These numbers provide an estimate of the volume occupied by each compartment. If a number of particles were randomly distributed within the cell, the number of particles counted for each compartment would equal the observed number of points, i. e. the observed points also represent an estimate for the expected number of particles.

Table 1. Synthetic data set in which nanoparticles enter cells by an endocytosis-independent mechanism and become randomly distributed between different intracellular compartments.

Compartment	Number of observed particles, N_o	Number of observed points, P	Number of expected particles, N_e	Relative deposition index, RDI	Chi-squared values
Cytosol	310	275	310.43	1.00	0.00
Nucleus	122	110	124.17	0.98	0.04
Mitochondria	59	50	56.44	1.05	0.12
RER/SER	27	25	28.22	0.96	0.05
Golgi complex	12	10	11.29	1.06	0.04
Phagosomes	32	30	33.87	0.94	0.10
Lysosomes/endosomes	19	15	16.93	1.12	0.25
Residual	6	5	5.64	1.06	0.02
Total	587	520	587	1.00	0.63

With 7 degrees of freedom, the total chi-squared value of 0.63 indicates that the null hypothesis (no difference between observed and expected distributions) must be accepted. Essentially, the particles are distributed randomly between cell compartments.

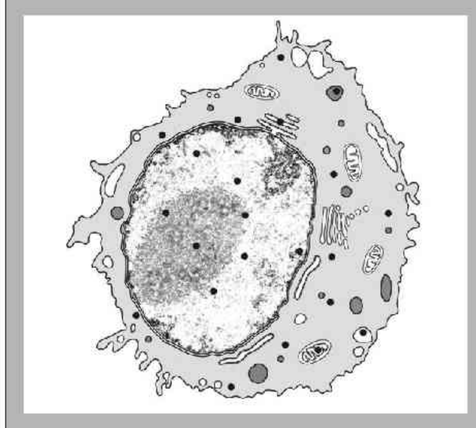

Figure 2.Random particle distribution (see Table 1). In this case, the particles are randomly distributed within the cell and the observed number of particles with each compartment is similar to the expected number of particles.

Table 2. Synthetic data set in which nanoparticles enter cells by an endocytosis-dependent mechanism and become distributed mainly in compartments of the endocytic pathway.

Compartment	Number of observed particles, N_o	Number of observed points, P	Expected particles, N_e	Relative deposition density, RDI	Chi-squared values	Chi-squared values as %
Cytosol	31	275	92.02	0.34	40.46	3.7
Nucleus	0	110	36.81	0.00	36.81	3.4
Mitochondria	0	50	16.73	0.00	16.73	1.5
RER/SER	0	25	8.37	0.00	8.37	0.8
Golgi complex	0	10	3.35	0.00	3.35	0.3
Phagosomes	94	30	10.04	9.36	702.25	64.0
Lysosomes/endosomes	42	15	5.02	8.37	272.47	24.8
Residual	7	5	1.67	4.18	16.96	1.5
Total	174	520	174	1.00	1097.39	100

With 7 degrees of freedom, the total chi-squared value of 1097.39 indicates that the null hypothesis must be rejected ($p<0.001$). Only two compartments have partial chi-squareds that contribute more than 10% to the total and have RDI values >1. These are the phagosomes and the lysosomes/endosomes.

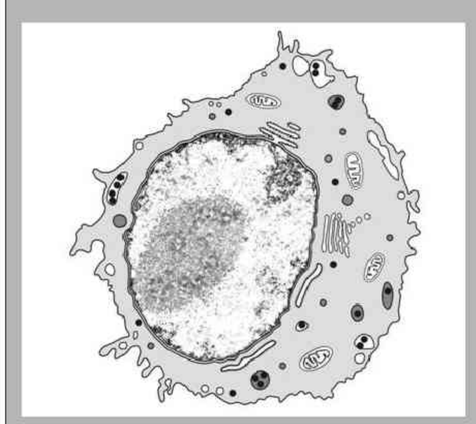

Figure 3.Non-random particle distribution (see Table 2). In this case, particles are predominantly located within phagosomes and endosomes/lysosomes. A few particles are also found in the cytoplasm. The observed number of particles in phagosomes and endosomes/lysosomes is higher than it would be expected from their size which results in a relative deposition index > 1. The observed number of particles for the cytoplasm is smaller than expected from compartment size leading to a relative deposition index < 1.

Application

Table 3. Important methodological issues of the experiments. For details see [2].

Animals	
Species	Adult male WKY/NCrl BR rats
Number	n=5 for each group
Particles	
Material	Titanium dioxide
Aerosol generation	Spark generator (Palas) in a pure argon plus 0.1 % oxygen stream
Count median diameter (nm)	22 (SD 1.7)
Exposure	Inhalation of aerosol for 1h
Fixation and tissue processing	
Time point of fixation	1h or 24h after particle exposure
Fixation mode	Subsequent perfusion fixation with 2.5 % buffered glutaraldehyde, 1 % Osmium tetroxide, 0.5 % uranyl acetate
Tissue sampling	Systematic uniform random sampling
Material for light and electron microscopy	Semithin (toluidine staining) and ultrathin (uranyl acetate and lead citrate staining) sections

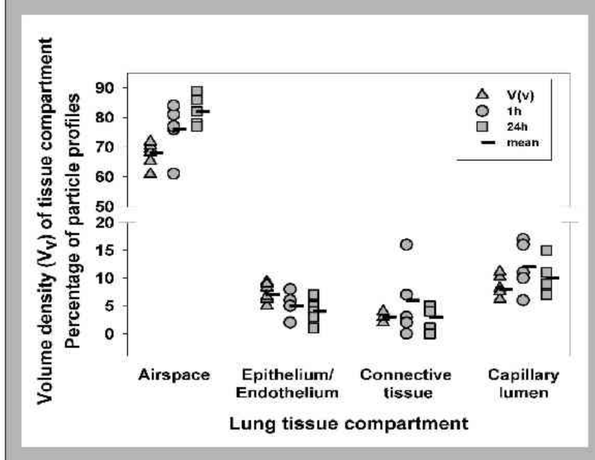

Figure 4.Original data from Geiser et al. [2] showing the mean number of TiO2 NP in the four defined tissue compartments at 1h and at 24 h after exposure. Volume fractions of the compartments are also shown. Reproduced from Geiser et al. [2]. With permission.

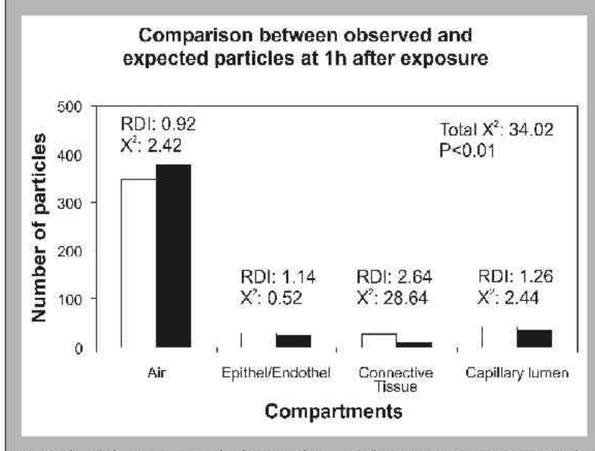

Figure 5.From the observed number of particles (N_O) and the number of points (P) on each compartment the expected number of particles (N_E)was calculated (e.g. N_E (air) = 449 x (2251/2672) = 378). The RDI is calculated from N_O/ N_E (e.g. RDI (air) = 348/378 = 0.92). The chi-squared (X^2) values are calculated from $(N_O - N_E)^2/N_E$ (e.g. X^2 (air) = $(348-378)^2/378$ = 2.42). With three degrees of freedom (2-1 groups x 4-1 compartments)and a total chi-squared value of 34.02, the null-hypothesis of random distribution has to be rejected ($p<0.01$). The connective tissue has an RDI of 2.645 and a partial chi-squared value that contributes about 84 % of the total chi-squared. It is the only compartment that meets both criteria for a preferential deposition. Abbreviations: Air: Air space; X^2: Chi-squared test values. RDI: Relative deposition index. that contributes about 84 % of the total chi-squared. It is the only compartment that meets both criteria for a preferential deposition. Abbreviations: Air: Air space; X^2: Chi-squared test values. RDI: Relative deposition index.

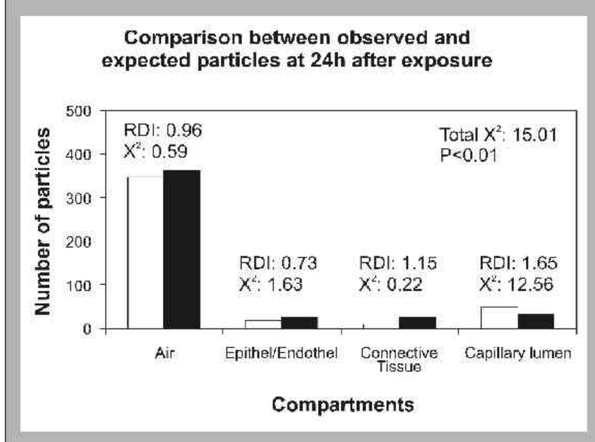

Figure 6.The calculation of the data is described in the Theoretical foundation and an illustrating example is provided in the note to table 4. With three degrees of freedom (2-1 groups x 4-1 compartments) and a total chi-squared value of 15.01, the null-hypothesis of random distribution has to be rejected ($p<0.01$). The capillary lumen has an RDI of 1.65 and a partial chi-squared value that contributes about 84 % of the total chi-squared. It is the only compartment that meets both criteria for a preferential deposition. Abbreviations: Air: Air space; X^2: Chi-squared test values. RDI: Relative deposition index.

References

[1] Oberdörster G, Oberdörster E, Oberdörster J (2005): Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ Health Perspect 113: 823-839. [2] Geiser M, Rothen-Rutishauser B, Kapp N, Schürch S, Kreyling W, Schulz H, Semmler M, Im Hof V, Heyder J, Gehr P (2005): Ultrafine particles cross cellular membranes by nonphagocytic mechanisms in lungs and in cultured cells. Environ Health Perspect 113: 1555-1560. [3] Mühlfeld C, Mayhew TM, Gehr P, Rothen-Rutishauser B: A novel quantitative method for analysing the distributions of nanoparticles between different tissue and intracellular compartments. J Aerosol Med, in press. [4] Mühlfeld C, Geiser M, Kapp N, Gehr P, Rothen-Rutishauser B: Re-evaluation of pulmonary titanium dioxide nanoparticle distribution using the "relative deposition index": Evidence for clearance through microvasculature. Part Fibre Toxicol, revised manuscript submitted.