Material Scientific Approach to Predict Nano Materials Risk of Adverse Health Effects

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Abstract. To estimate the potential risk of nano materials, correlations were investigated between material properties and various biomarkers indicating adverse effects on humans. Nano materials have a variety of properties such as solubility, iso-electric point, crystal shape, BET specific surface area and so on. The purpose of our work was to predict relationships between material properties and hazard data by undertaking statistical survey of eleven papers arguing cell viability assays.

The reviewed papers associate cytotoxicity (i) mainly with particle volume and (ii) a certain degree with particle solubility, with relatively large variability of toxicological responses. At present nanomaterials are often very broadly named, defined and categorized based upon only their chief chemical composition or product shape – e.g., “titanium,” “carbon black,” “nano tubes,” etc. Such rough, imprecise categorization serves little or no useful purpose when attempting risk assessments for every nano material produced differently, since even materials with the same name can possess different properties and consequently different degrees of hazards.

1. Introduction

More than 300 nanotechnology products such as sunscreens or semiconductors are already on the global market [1]. Supported by government funding, multiple nano-related research projects are being carried out at an unprecedentedly rapid pace. However, many companies have come to withdraw from such research because of the potential adverse effects or uncertain risks [2]. We are trying to develop risk assessment software which can suggest a clear direction of controlling manufacturing processes to support nanotechnology developers.

The concepts are shown in Figure 1. Nano materials possess various properties: specific surface area, zeta potential, solubility, crystal type and so on. It is well known that the diameter or surface area of nano materials correlates highly with inflammation response. In this study, we focused on other parameters and found correlations. We will examine another and attempt to predict whether nano materials could invade into the human body or how they transfer inside the body.
2. Methods

2.1. Researching papers
For a viability analysis of various types of particles, many papers were searched and selected by using the ISI Web of Knowledge (v4.4). The selected papers consisted of experiments involving cell viability assays performed in vitro with nano/sub-micro/micro particles of different chemical species. In the experiments, various types of cells (fibroblasts, osteoblasts, etc.) except macrophages were used, and the endpoint was set as the viability of cells after 3 days exposure.

2.2. Dose-mortality analysis in vitro (size effect)
From the experimental results reported in the selected papers dose and viability data sets were singled out, and scatter diagrams were made with dose as the horizontal axis and “% death” as the vertical axis. “% death” means mortality rate of cells exposed to particles versus unexposed cells calculated by “100 (%) - % of viable cells”. Dose was varied by using 4 unit types. “µg/ml” is a unit for particle weight over cell culture media volume. “cm$^2$/ml” is in one way, a unit for particle surface area computed by BET measurement over media volume, and, in another way, a unit for particle surface area calculated from particle’s minor and major axes. When a particle has a spherical shape, calculated surface area (m$^2$/g) should be $6/\rho D$ where $\rho$ (g/cm$^3$) is density and $D$ (µm) is diameter. If a particle is fibrous, calculated surface area should be nearly $4/\rho D$ if $D$(minor axis) $<<$ major axis. “No. of particles” is a unit for particle count over media volume. In addition, in the scatter diagrams, each plot had different marks depending on size: one type of key for particles with a primary diameter less than 120 nm and another key for those above 400 nm. Generally “nano-sized” is said to be 1-100 nm, but in this paper the collected data were distributed in 1-120 nm and 400-10 000 nm.

2.3. Dose-mortality analysis in vitro (particle species effect)
For detecting the chemical species effect or crystal shape effect, the scatter diagram plots above were given different marks depending upon particle species. Here the particle species were identified using...
chemical composition and particle crystal shape. In addition, regression lines (Y = a + b ln(x)) were drawn for each particle species, and EC50 was evaluated. EC50 was calculated as the concentration of µg/ml needed to reduce cell proliferation to 50% of unexposed controls.

2.4. EC50 comparison between particles (solubility effect)
To study the correlation between particle solubility and cell mortality, an x-y scatter diagram was produced with the x-axis showing particle solubility and the y-axis calculated EC50. Solubility data were obtained from “Chemistry Handbook basic edition 2, 4th revised edition”. All table calculations or analyses were performed using Microsoft Office Excel 2003.

3. Results
Eleven Papers [3-13] discussing in vitro experiments concerning the viability of cells except macrophages were selected. These papers checked the endpoint of cell viability after 3 days exposure. If BET surface area was not described in a paper, calculated surface area was substituted for BET surface area (On spherical particle, calculated surface area (m²/g) should be 6/ρD. On fibrous particle, that should be nearly 4/ρD if D (minor axis) << major axis). The collected cell viability data were used to make scatter diagrams. Figure 2(a) shows a conventional diagram whose horizontal axis is particle dose (µg/ml) and whose vertical axis is %death (100 (%) - viability (%)). Its keys were differentiated in particle diameter between 0-120 nm and 400-10 000 nm. Plots of 0-120 nm and 400-10 000 nm looked well overlapped so that the effect of size on cell viability is likely to be indicated by particle weight or volume (since particle weight and volume show similar values). Figure 2(b) indicates a diagram whose x-axis is the particle dose of BET surface area (cm²/ml). There seems to be a split between the 0-120 nm group and the 400-10 000 nm group. Figure 2(c) has an x-axis as the particle dose of calculated surface area (cm²/ml). Here BET surface area of amorphous silica was converted to the smaller value of calculated surface area, but the whole trend of scattering plots was not differed notably. Figure 2(d) has an x-axis as the particle dose of the number of particles (No. of particles/ml). Two groups were split more widely in two and the size effect for cell viability is not likely to be number of particles.

The collected cell viability data are all shown in Figure 3 with each type of mark representing a particle species. Additionally, a regression line Y = a + b ln(x) was drawn on each particle species and EC50 (the concentration of µg/ml that reduced cell proliferation to 50% of unexposed controls) was evaluated.

Seen as Figure 4, an x-y scatter diagram was developed, with the x-axis particle solubility (g/l) and the y-axis EC50 (The solubility of carbon materials was given as 10^{-10} g/l as a matter of practical convenience). There is a possible correlation: the greater solubility, the smaller the EC50 (meaning greater cytotoxicity). The plot for TiO₂ (anatase) looks distant from the correlation and there may be other, more important factors affecting their cytotoxicity (i.e. oxidative stress caused by surrounding visible or ultra violet light), or the data volume may have simply been insufficient.
4. Discussion

To study the effects of particle size and species for hazard assessment, dose-mortality analyses in vitro were performed on this paper. Results suggest that the size effect on cytotoxicity is ascribed to particle weight or volume and that another cytotoxic factor is particle solubility.

The effect of particle size on cytotoxicity has been argued in many papers. Nano and micro alumina and titania particles was tested by viability assays, and viable osteoblast density was not always smaller in nano particle cultures than micro particle cultures when particle weight dose (µg/ml) was equivalent [14]. This trend is also seen in Figure 2 of our results showing data of additional particle species and more plots. Another paper argued that cytotoxicity is not dependent on size or chemical species but on total volume [11]. The authors introduced five species of ceramic particles into cell culture and measuring the dose-response curve with varying doses using three criteria: particle number, surface area, and volume. Their results were quite similar to ours in splitting formation of plots on scatter diagrams. On the other hand, the cytotoxic effect of aggregated nano materials was investigated, with little correlation found between BET specific surface area and the cytotoxicity index [15]. Our results also support it and possibly the order of cytotoxicity is explained by solubility.
Accordingly, the chief factor for cytotoxicity appears to be the particle volume dose and subordinate factor may be particle solubility. A possible mechanism could be that particle volume obstructs intra-cellular adherence or cell-extracellular matrix interaction and slows cell proliferation. Here, equivalent particle surface area dose does not cause equivalent mortality because smaller particles are more susceptible to being pushed away. Additionally, dissolved materials may affect cellular osmotic systems or increase reactive oxygen species.
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