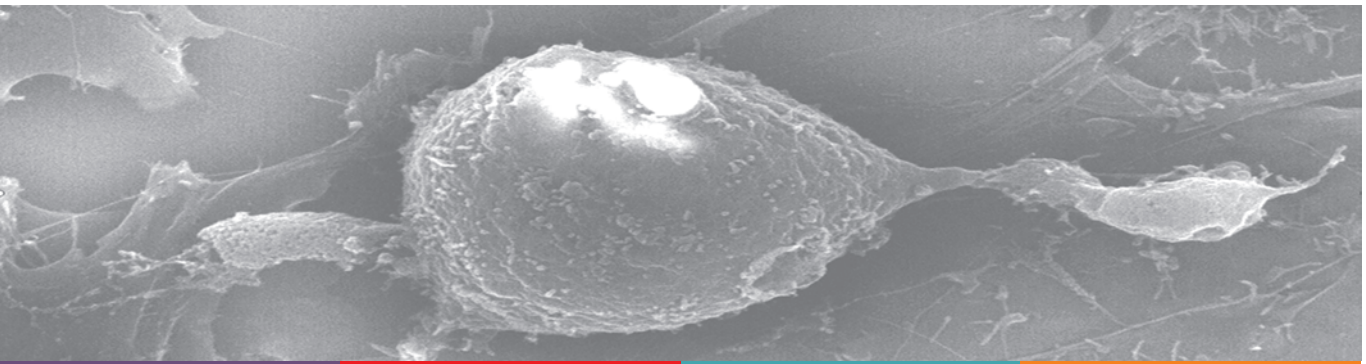
Cell_Nano_Tox

Cellular Interaction and Toxicology with Engineered Nanoparticles



Cellular Interaction and Toxicology with Engineered Nanoparticles

www.fp6-cellnanotox.net

SIXTH FRAMEWORK PROGRAMME

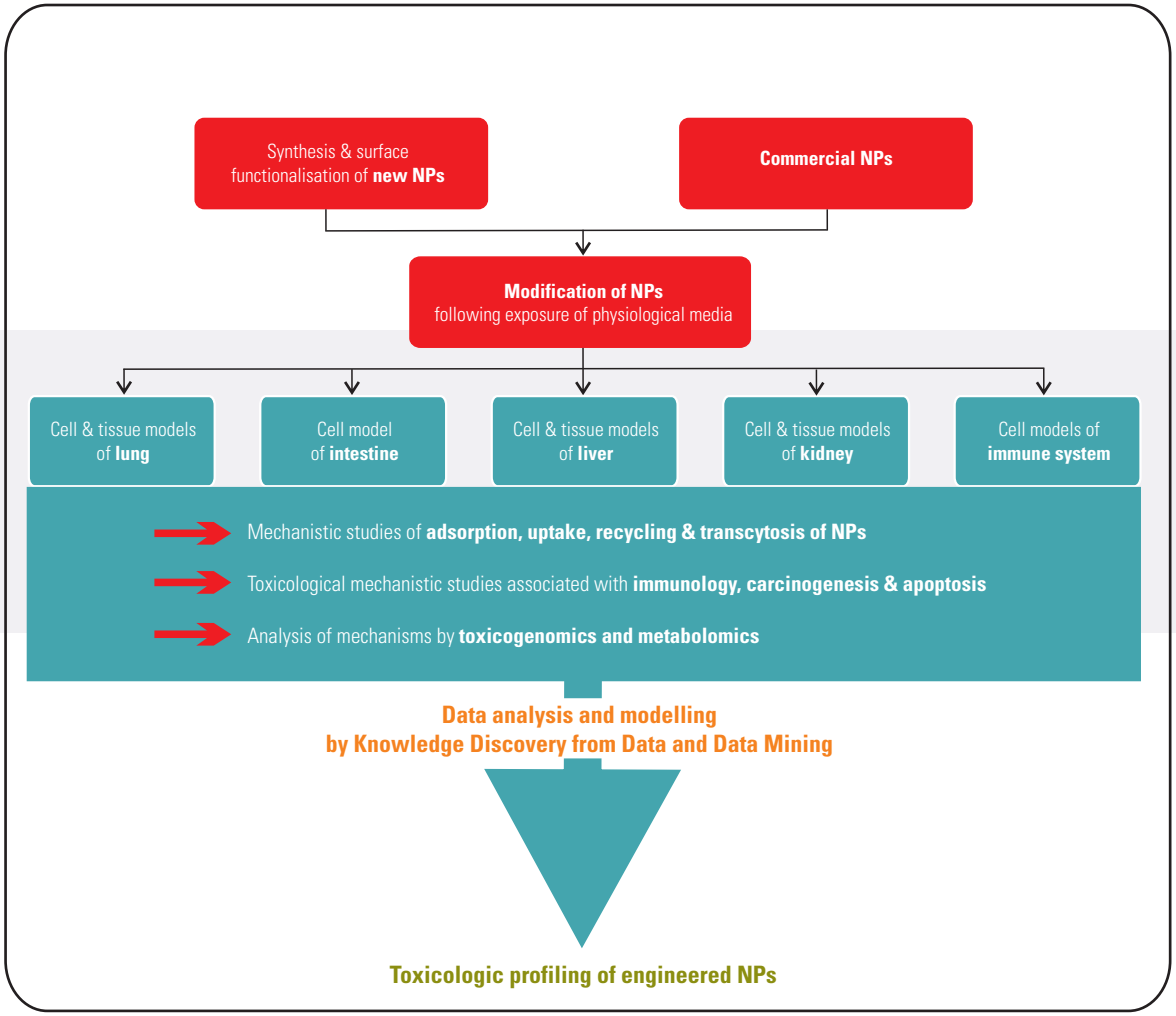
CellNanoTox Concept

Toxicological profiling of engineered nanoparticles

CellNanoTox aims at the development of an innovative multidisciplinary set of indicators for determining the toxicity of NPs and unravelling the correlation between the physicochemical characteristics of NPs and their toxic potential on different organs of the human body.
For a comprehensive understanding of the complex data obtained on toxicology of NPs, based on in-vitro and ex-vivo studies, we will employ conventional toxicology combined with toxicogenomics and metabolomics. Knowledge Discovery from Data (KDD) and Data Mining (DM) methodologies will be developed for the specific NP toxicological datasets obtained.

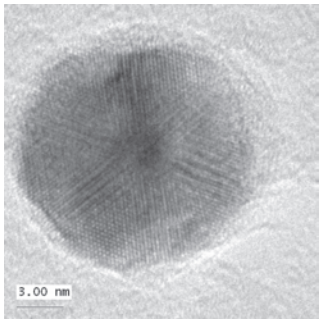
In **CellNanoTox** highly specialised industrial and academic know how and technologies will be integrated for the synthesis and analysis of NPs as well as for the study of NPs' interaction with different cell and tissue models.

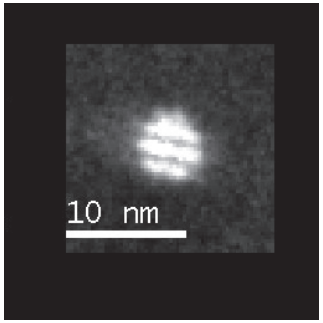
CellNanoTox addresses the need of European society for assessing the risk of occupational and general population exposure to industrially manufactured NPs. It will generate new knowledge on potential health risk or the absence of it, providing objective arguments for recommendations and regulations.

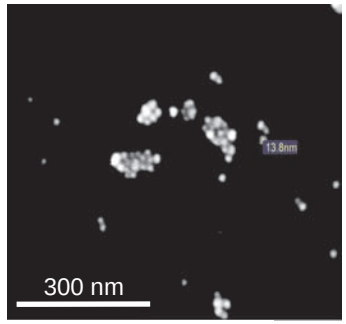


Cover picture: Cobalt aggregates in immortalised mouse fibroblast (Balb/3T3 cell) by Scanning Electron Microscope (SEM-EDX). Cells were exposed for 24h to 10μM, dehydrated and treated using Critical Point Dryer technique (Joint Research Centre)

NP-Synthesis and Characterisation







State-of-the-art

Although very important results have been attained through different NP-synthesis strategies, the total control of NPs' chemical composition, average size and surface state is still difficult to obtain. Additionally, related to toxicology, the main problem is the difficulty of sensitive localisation of NPs in cells when employing light microscopy.

Introduction of NPs into aqueous media may either result in a stable dispersion or may lead to their agglomeration. Furthermore, the surface characteristics of the NPs may be altered once the NPs interact with human body fluids. This is particularly the case when NPs penetrate into the blood circulation. The in-vitro studies require the addition of the NPs to cell and tissue culture media or serum. NPs can release biologically active ions (e.g. Co, Zn) in the case of composite NPs, or may be coated by proteins of the culture media or the serum. The current methods for detection of free ions by colorimetric methods or by atomic absorption are restricted to relatively high concentrations.

NP-Synthesis and Characterisation

Synthesis of NPs possessing different chemical composition, size, and chemical surface state, and labelled by radioactive isotopes and fluorescence probes

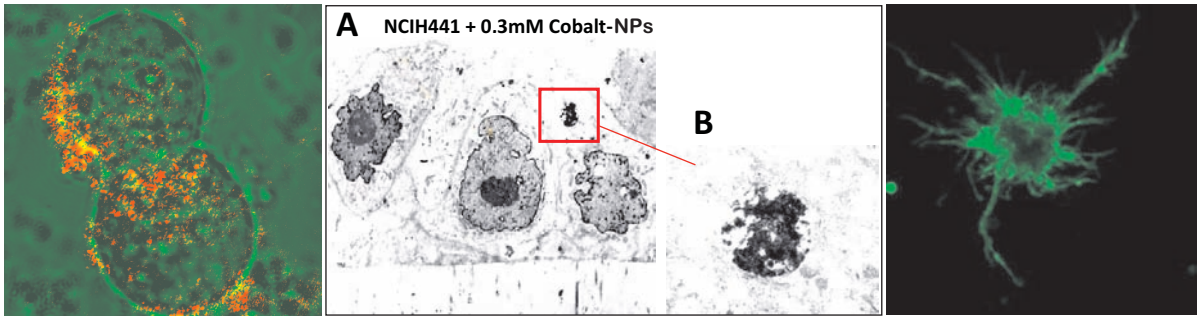
Obtaining a preset NPs' chemical composition, average size, and surface state control is in itself a challenging task. In particular, polyol method will be used to allow production of NPs' dispersions in biocompatible solvents (glycols) of non-aggregated organic coated and uncoated NPs. The introduction of organic bi-functionalised molecules will enable the study of the effect of defined surfaces on toxicology. A major innovation will be the possibility of radiolabelling the product without affecting the particles' chemical and other properties. Organic fluorescent labelling will also be used for spatial and temporal detection of NPs in cells.

Adsorption of physiological substrates to the surface of NPs and ion release from NPs

The sole way to detect very minute amounts of free metal released is to use radioactive isotopes of the metal. This requires the formation of radioisotopes in the raw metals followed by synthesis of simple and complex NPs from these isotope containing raw materials. The detection of the type of proteins adsorbed to the NPs can in principle be carried out by applying a battery of antibodies against different proteins present in a serum. Since this is a rather complex procedure, we intend to use an approach based on mass spectrometry technologies.

Pictures: High resolution image of a quantum dot (WWU Münster) - Dark field TEM image of a twinned Cobald Ferrite Nanoparticle (WWU Münster) - SEM of Gold NPs agglomerates dispersed in water (Colorobbia)

Nano-Bio-Interaction



Interaction of NPs with living systems

Like many toxicological agents, NPs are liable to interact adversely with cellular metabolism in liver, kidneys and lungs. But, until now, such interactions remain largely unknown. In most studies, the interaction of toxicological agents with energy and intermediary metabolism is classically evaluated by measuring a small number of simple metabolic parameters. This provides only a restricted view of metabolic pathways. In addition, the maintenance of metabolic differentiation of lung slices remains to be established.

Dendritic cells of the immune system are in the first line of defence of the body, they are known to endocyte particles, are activated by foreign molecules and then induce immune responses. Several studies demonstrated that some NPs such as carbon nanotubes interfere with cells of the immune system.

In order to reduce in-vivo assays using animals in the future, it is necessary to set up a series of in-vitro assays based on cell models. In functional studies, although cell lines may provide interesting information, it is essential to develop assays using non transformed cells such as primary cultured cells as a first step.

In-vitro modelling of NPs' interaction with lung and lung toxicology

In addition to cell lines, allowing high throughput screening, primary in-vitro cell culture models of the human alveolar epithelium possessing the relevant qualities of the alveolar epithelium will be used to study the effects of NPs in-vitro. Lung slices still have the structure of the lung and contain all cell types. The use of this organ model might enable the identification of relevant biological parameters involved in lung toxicity of NPs.

Nano-Bio-Interaction

Interaction of NPs with liver, kidney and lung metabolism

A panoramic view of all the pathways involved in the metabolism of physiological substrates (glucose, lactate, glutamine) will be obtained in liver, kidney and lung slices thanks to the use of metabonomics to which we will combine enzymatic, radioactive and carbon 13 NMR spectroscopy measurements along with mathematical modelling of metabolic pathways and bioinformatics. This will enable identification of the enzymatic steps affected by NPs.

Interaction of NPs with the immune system

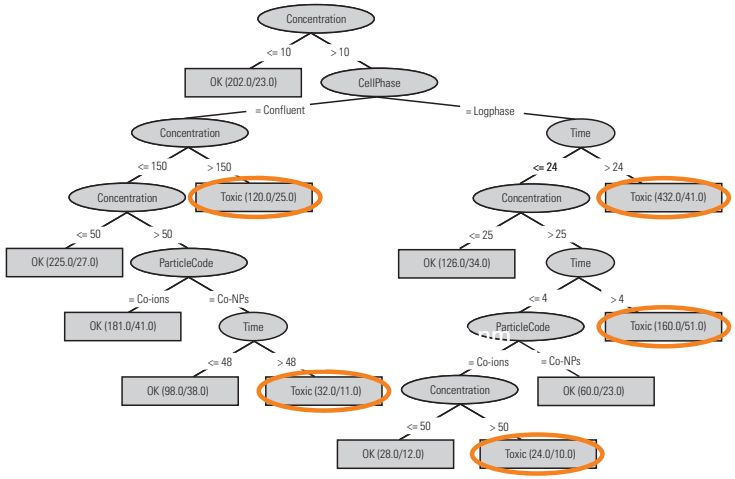
Dendritic cells from mouse bone marrow will be exposed to NPs, and then cell viability and activation of the cells will be monitored by flow cytometry using specific markers. The presence of NPs in the endocytic pathways of the dendritic cells will be followed up by fluorescence and confocal microscopies.

Interaction of NPs with complement system of the blood

NPs, as other foreign particles, may induce activation of the complement system (C') present in blood serum or plasma. This will be assayed by in-vitro biochemical assay. We shall provide complete information about properties of the studied NPs: first by identifying C' protein bound to their surfaces and their biological consequences; and second by defining modification of NPs' shape by high resolution microscopy.

Pictures: Uptake of 15 nm gold NPs by cells using reflection mode of confocal microscopy and antiflex optics (Tel Aviv University) - Transmission electron microscopy of the human alveolar Type II NCI-H441 cell line exposed to 0.3mM cobalt nanoparticles (Co-NPs) (A). Panel B is a magnification of the selected area highlighted in panel A (Johannes Gutenberg University)- Modification of class II molecules localization during dendritic cell activation (INSERM)

Knowledge Discovery



Knowledge Discovery of Data & Data Mining

In studying toxicological effects of NPs there will be specific data, which will require new algorithms (or enhancements based on available methods) in order to provide meaningful compact models for gaining insight into the phenomena and their most important attributes.

Knowledge Discovery from Data (KDD) is an automatic, exploratory data analysis and modelling of large data sets, such as those ones that will be available through the research on toxicological effects of NPs.

KDD is the organized process of identifying valid and novel models. Data Mining (DM) is the core of the KDD process, involving inferring algorithms that explore the data, develop the model and discover previously unknown patterns. There are numerous recent references to KDD and DM which discuss generalized error of concluding from limited data sets. In CellNanoTox KDD will be used to support the investigation on toxicological effects of engineered NPs.

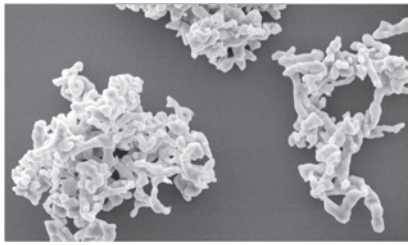
Knowledge Discovery

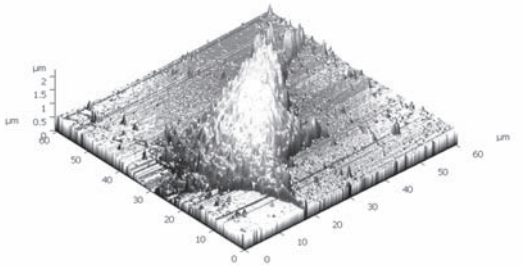
One objective of CellNanoTox is to apply the discipline of Knowledge Discovery from Data (KDD) and Data Mining on the database that will be set up during the project duration and made available to the stakeholders beyond the project duration. The knowledge discovery process is iterative and interactive, consisting of nine steps. The process starts with determining the KDD goals, and ends with the implementation of the discovered knowledge.

1. *Developing an understanding of the domain of NP toxicology:* definition of the goals, determination of the data to be used for the KD.
2. *Selecting and creating a data set* on which discovery will be performed: finding out available data, obtaining additional necessary data, and then integrating all the data for the knowledge discovery.
3. *Preprocessing and cleansing:* enhancing reliability of the data, handling missing values and removal of noise or outliers.
4. *Data transformation:* generating better data for the data mining.
5. *Choosing the appropriate strategy:* taking into account the level of meta-learning and decomposition related to the particular set of available data.
6. *Choosing the Data Mining Algorithm:* selecting the specific method to be used for searching patterns.
7. *Employing the Data Mining Algorithm:* performing the data mining algorithm, considerations of actual memory and interim results.
8. *Evaluation:* evaluation and interpretation of the mined patterns (rules, reliability etc.).
9. *Using the discovered knowledge:* usage and overall feedback on the patterns and discovery results obtained by data mining.

Picture: Discovering rules of toxic nano particles (NPs) (Tel Aviv University)

Background





CellNanoTox project aims at the development of an **innovative multidisciplinary set of tests and indicators for toxicological profiling of nanoparticles (NPs)** that will be based on an interdisciplinary approach combining inorganic and organic chemistry, physiology, toxicology, cell biology and data analysis:

- **Design and synthesis of coated and non-coated NPs** of industrial importance, radioactive and fluorescent labelled, of various chemical composition, possessing different size and surface characteristics.
- **Characterisation** of dispersion and modification of surface characteristics of the NPs following interaction with physiological media.
- Elucidation of interaction and mechanisms of **uptake and recycling of NPs by in-vitro cellular model systems** of lung, intestine, liver, kidney and the immune system.
- Establishment of an **in-vitro model of the lung** and in-vitro evaluation of transport mechanisms through the lung-vascular and intestinal cells with respect to NPs characteristics.
- Determination of the NPs-induced toxicology and underlying mechanisms in in-vitro model systems of lung, intestine, kidney, liver and the immune system by conventional **toxicology and toxicogenomics.**
- Elucidation of NPs-induced metabolic changes in precision-cut slices of lung, liver and kidney by **metabolomics.**
- Study of the activation and the **inflammatory response** of the immune system towards NPs. Development of new approaches to study the **interaction and induced effects of NPs with in-vitro cell/tissue models.**
- Development and adaptation of novel methodologies of **Knowledge Discovery from Data (KDD) and Data Mining (DM) for toxicological studies.**

Background

Pictures: Aggregates of cobalt nanoparticle by Scanning Electron Microscope (SEM-EDX). Cobalt nanoparticles (mean size < 50nm) are not coated and form aggregates in powder state and in suspensions with other solvents e.g. water, culture media (Joint Research Centre) - Morphological analysis by Atomic Force Microscope technique of immortalised mouse fibroblast (Balb/3T3 cell) (Joint Research Centre)

Background

Nanotechnology will lead to a variety of innovations in fields such as:

- Medical applications (e.g. diagnostics, tissue engineering, drug delivery)
- Information technologies (e.g. data storage)
- New materials (e.g. surface coating)

Several nanotechnology-based products have been marketed such as medical products, paints, creams. The market for such products is estimated to be currently ~ 2.5 billion € and could rise to hundreds of billions by 2010 and one trillion thereafter.

For the rapidly evolving technology it is important to ensure safety at the earliest possible stage.
Therefore it is necessary to undertake appropriate R&D to provide data and methodologies for toxicology in order to enable risk assessment.

Ref.: EC's publication
'Towards a European Strategy for Nanotechnology'

CellNanoTox Partners

Tel Aviv University
Department of Physiology and Pharmacology of the Faculty of Medicine
Tel Aviv, Israel
Prof. Dr. Rafi Korenstein
CellNanoTox coordinator
Mechanistic aspects on adsorption and uptake of NPs by cells

Tel Aviv University
Department of Industrial Engineering of the Faculty of Engineering
Tel Aviv, Israel
Prof. Dr. Oded Maimon
Data Mining and Knowledge Discovery

Joint Research Centre
Institute for Health and Consumer Protection, Nanotechnology and Molecular Imaging Unit
Ispra, Italy
Dr. Francois Rossi
Dr. Jessica Ponti
In-vitro toxicology, NPs synthesis and characterization by physic-chemical methods (XPS, ToF SIMS)

INSERM
Institut National de la Santé et de la Recherche Médicale
U 820: Métabolomique et Maladies Métaboliques
Lyon, France
Prof. Dr. Gabriel Baverel
Cellular Metabonomics, NMR spectroscopy, NPs interaction with liver, kidney and lung

U 823: Immunologie Analytique des Pathologies Chroniques
Grenoble, France
Dr. Patrice Marche
Dr. Christian Villiers
Immune Response on NPs, dendritic cell biology, NPs interaction with biological fluids

CellNanoTox Partners

Coordinator:

Tel Aviv University
Department of Physiology and Pharmacology of the Faculty of Medicine
The Marian Gertner Institute for Medical Nanosystems
Tel Aviv, Israel
Prof. Dr. Rafi Korenstein
www.fp6-cellnanotox.net

Westfälische Wilhelms University of Muenster
Institute of Mineralogy
Muenster, Germany
Dr. Ute Golla-Schindler
Dieter Sommer
NPs analysis by electron microscopy and mass spectroscopy

Johannes Gutenberg University of Mainz
Institute of Pathology
Mainz, Germany
Prof. Dr. C. J. Kirkpatrick
Dr. Chiara Uboldi
In-vitro study on NPs-cell/biomaterial interaction, effect on alveolar and endothelial cells

BASF SE
BASF Product Safety - Regulations, Toxicology and Ecology
Ludwigshafen, Germany
Dr. Robert Landsiedel
Susanne Boehn
NPs interaction with lung

Colorobbia Italy Spa
CERICOL
Vinci, Italy
Dr. Giovanni Baldi
Dr. Daniele Bonacchi
NPs synthesis, functionalisation, modification

tp21 GmbH
Management
Saarbruecken, Germany
Dr. Petra Zalud
Dr. Hanno Wittig
Project Management, Public Relations

Disclaimer

The information in this document is provided as is and no guarantee or warranty is given that the information is fit for a particular purpose. The user thereof uses the information at its sole risk and liability. The STREP Project no. FP6-2004-NMP-TI-4- 032731 CellNanoTox has received research funding from the Sixth Framework Programme of the European Community. Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the given information. *August 2008*

Imprint:

Published by CellNanoTox consortium, coordinated by Tel Aviv University, IL

Concept and realization: tp21 GmbH, Saarbruecken, D
Graphic Design: Prof. Wolfgang Schabbach, Saarbruecken, D
Printed by: Ottweiler Druckerei und Verlag GmbH, D

Specific Targeted Research Project no. FP6-2004-NMP-TI-4- 032731
funded by the European Commission under the Sixth Framework Programme