Cell_Nano_Tox
Cellular Interaction and Toxicology with Engineered Nanoparticles

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SIXTH FRAMEWORK PROGRAMME

Specific Targeted Research Project no. 032731

Thematic Priority: NMP-TI4

Publishable final activity report

Reporting Period (42 months):
November 01 2006 - April 30 2010

Start date of project: **November 1st, 2006**

Project duration **42 months**

Date of document preparation: **May 2010**

Rev. [1]

Project coordinator name:

Prof. Rafi Korenstein

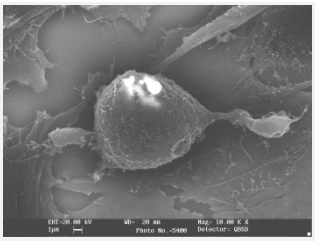
Project coordinator organisation name:

Tel Aviv University

1. Project execution

CellNanoTox aims at the development of innovative multidisciplinary sets of tests and indicators for toxicological profiling of nanoparticles (NPs) as well as unravelling the correlation between the physicochemical characteristics of NPs and their toxic potential on various organs of the human body.

For a comprehensive understanding of the complex data to be obtained on toxicology of NPs, based on in-vitro and ex-vivo studies, we have employed conventional toxicology combined with the methodologies of toxicogenomics, metabonomics, Knowledge Discovery from Data (KDD) and Data Mining (DM).



Cobalt aggregates in immortalised mouse fibroblast (Balb/3T3 cell) by

Scanning Electron Microscope (SEM-EDX).

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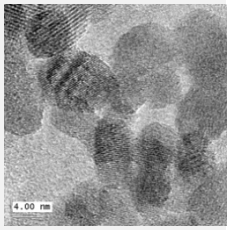


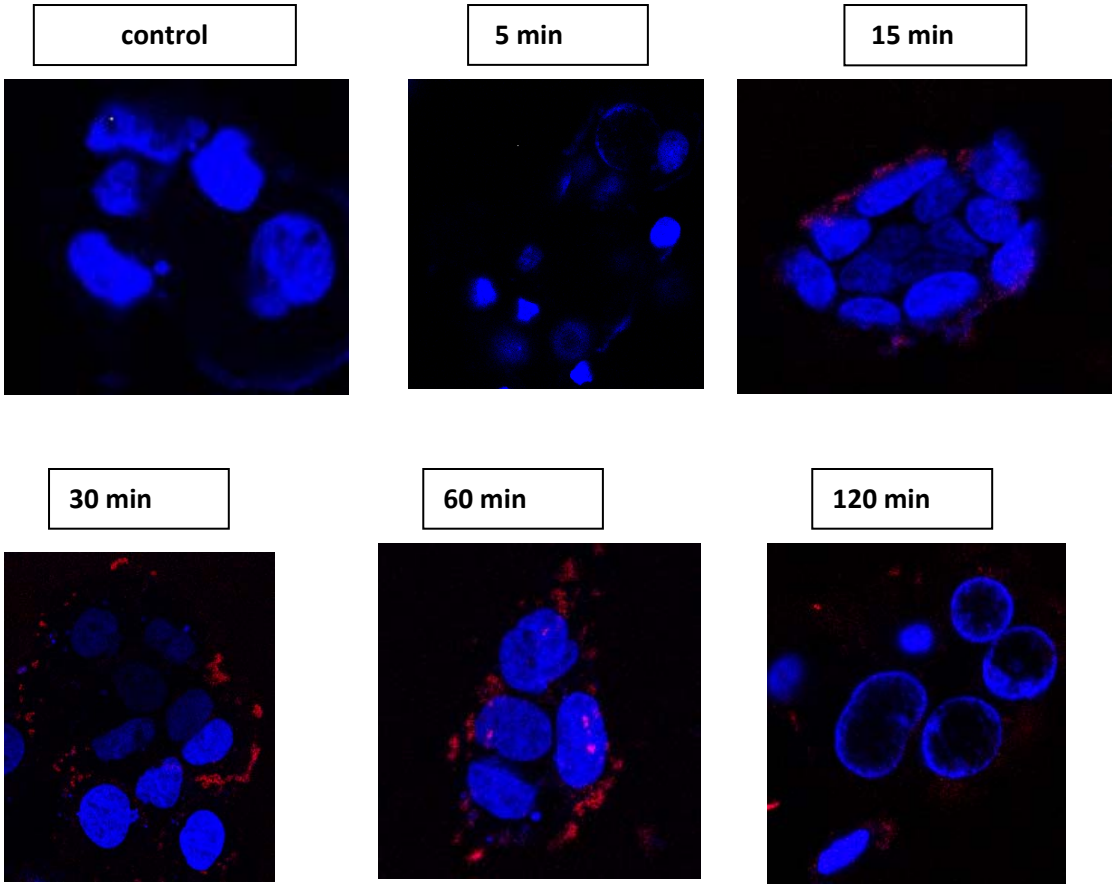
Cobalt Ferrite Nanoparticles by TEM

(copyright WWU)

This project is focused towards understanding the relation of size and surface chemistry on the deposition, uptake, translocation, and toxicity of a few selected industrially important NPs as well as novel synthesized NPs, whose size and surface chemistry will be methodically modified. Since it was shown that the penetration of NPs into the human body proceeds principally through inhalation or orally, whereas penetration through healthy skin is restricted, we have chosen lung and intestine as the primary interacting tissues/organs with NPs, while liver, kidney and the immunological system have been selected to be the secondary major sites of interaction, following the penetration of NPs into the blood circulation. The interaction of the NPs with these different target organs has been studied by making use of alternative

methods to animal experimentation by employing in-vitro cell systems as well as ex-vivo studies based on precision-cut slices of lung, liver and kidney. During the project period we have performed toxicological screening of some commercial and non-commercial nanoparticles. This has been done using different cellular models of lung, intestine, liver, kidney and cells of the immunologic system. The various cellular systems showed somewhat different susceptibility towards the exposure to the nanoparticles, though the overall trend of the toxicological response was similar. Under the concentration range used, gold nanoparticles have shown almost no toxicity whereas cobalt aggregates of nanoparticles, cobalt ferrite and quantum dots were shown to be toxic. The toxicological response depended on the cellular model as well as on the duration of exposure to the nanoparticles. Longer exposure time has resulted higher toxicity than a short one. Large part of the observed toxicity could be attributed to the effect of ions which leached from the nanoparticles into the extracellular millie. One of the consequences of being exposed to nanoparticles was an oxidative stress imposed on the cells and inflammantory response of the cells the NPs. We have also found that the nanoparticles are internalizing the different cells, by different methods: (i) EM pictures of gold and cobalt ferrite NPs; (ii) Quantitive uptake into the cells of radiolabled gold and cobalt ferrite NPs; (iii) Tracking fluorescence coated cobalt ferrite NPs and Quantum dots inside the cells by confocal microscope and by flow cytometry. We have employed metabolomics technique on liver and kidney thin slices**,** Additionally we have studied in-depth immunological response of dendritic cells to the different NPs and Finally we have applied the KDD and data-mining methodology in the analysis of the toxicity of cobalt NPs Co-ferrite using different cellular models.

The studies carried out within the CellNanoTox project address the needs of the European society for assessing the risk of occupational and general population exposure to industrially manufactured NPs. It is expected generate new knowledge on potential health risk or the absence of it, providing objective arguments for recommendations and regulations.



Pictures:

Confocal microscopy images of adherent Caco-2 cells incubated at 37ºC with 8 µM carboxyl-QDs at different time intervals. QDs (red)– EM: 800 (spectra was confirmed each time at the red dots), Hoechst stain (blue). Observed by confocal Zeiss LSM 510 40X, 60X objectives, live cells.

Methodologies employed and the achievements of the project

1. Modifying toxicological screening methods for the specific NPs: MTT assay, Alamar blue assay, protein determination, neutral red assay – We have adjusted some protocols for standardisation of toxicity assays in different cellular models in order to get the most reliable and correct results.

2. Synthesis of newly NPs: We have succeeded in the synthesis of newly interesting NPs, such as quantum dots, gold, cobalt ferrite, labelling radioactively gold and cobalt ferrite NPs.

3. Performing quantitative uptake experiments by radioactivelables NPs - The common way to assess quantitatively the intracellular distribution of non-fluorescent NPs is by transmission electron microscopy. However, this method requires a large effort of quantitative image analysis and the employment of serial sectioning, therefore exploring the radioactive entrance of NPs in the cell can replace partially EM method.

4. Modifying toxicological methods for standardisation of toxicity assays in thin lung and liver slices - We have adjusted some protocols (LDH release, WST assay (similar to MTT assay) in order to get the most reliable and correct results, additionally the establishment of an alternative method is important by means of reduction in animal numbers used for toxicological experimentation, precision cut slices of rat lung can be a valuable tool.

5. Employing metabolomics in the studying of thin liver and kidney thin slices - Metabonomics is defined as “the quantitative measurement of time-related multiparametric metabolic response of living systems to pathophysiological stimuli or genetic manipulations”. Proton NMR spectroscopy is frequently used as an analytical tool, and it is currently being applied to biological fluids, mainly urine and blood plasma or serum. Its objective is to identify metabolic biomarkers specific to organ disturbances under *in-vivo* situations.

6. Studying the immunological response towards the selected NPs in dendritic cells by specific immunological methods - we have assayed the activation of the immune system and characterized patterns of inflammatory molecules produced in response to exposure to NPs.

7. The KDD and data-mining methodology was applied in the analysis of the toxicity of cobalt NPs and Co-ferrite NPs using different cellular models - Knowledge Discovery from Data (KDD) is an automatic, exploratory data analysis and modeling of data sets. 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 - by studying toxicological effects of NPs with specific data, we could provide meaningful compact models for gaining insight into the phenomena and its most important attributes.

High throughput toxicological screening of selected NPs

We have carried out toxicological screening of four family types of NPs consisting of cobalt NPs, gold NPs, cobalt-ferrites NPs and quantum dots, using in-vitro model systems of lung, intestine, liver, kidney and the immune system. Gold NPs, cobalt ferrite NPs, as well as corresponding radioactively labeled NPs were synthesized by the consortium. The screening was based on alveolar type II cells and lung-slices for lung, on Caco-2 cells for intestine, on MDCK and HEP-G2 cells for kidney and liver respectively and on murine primary dendritic cells for the immune system. Under the concentration range used, gold NPs and quantum dots were shown to be non-toxic whereas aggregates of cobalt-NPs and cobalt-ferrite NPs were shown to be toxic.

Dose-response curves of cobalt NPs aggregates were examined employing MTT, neutral red (NR) and Alamar blue assays. Since cobalt NPs undergo dissolution in aqueous media, we determined the dose-response curves for Co-ions, employing cobalt chloride for the same endpoints. The extent of cobalt NPs dissolution was determined to enable us to examine the indirect effect of cobalt NPs on the various cell-types due to dissolution as compared to the direct effect of aggregated cobalt NPs on the different cell models. Data analysis and modelling of the obtained data sets, for the toxicological dose-response curves for cobalt NP aggregates, taking into account the dose-response curves of cobalt ions, was carried using the approach of Knowledge Discovery from Data (KDD). The first KDD goal was to discover rules for determining the toxicity of nanoparticles from the experimental results. The input data set included the consolidated experimental results, where each data record has the following attributes: (1) Cell type; (2) Particle type (Co-ions or Co-NPs); (3) Concentration; (4) Exposure time; and (5) The extent of viability decrease.

The observed toxicity was modelled by a J48 decision tree classifier since such classifier model can be explained intuitively, in. Thus, concentration is the most influential parameter (highest rank), as expected from the basic principles of toxicology. The second most influential parameter (second rank) is either the compound type (Co-ions or Co-NPs) or the cell type, depending on the concentration range. The third and the lowest rank in the model is that of the duration of exposure.

This model is pointing at the differential sensitivity towards toxicity of the different cell lines for cobalt ions and cobalt NPs. The hierarchy of cell sensitivity towards cobalt ions is given in the following sequence: A549 > MDCK > NCIH441 > Caco-2 > HepG2 > Dendritic cells, where A549 is the most sensitive cell line and primary dendritic cells are the least sensitive ones. However, a different hierarchy pattern emerges for Co-NPs: A549 = MDCK = NCIH441 = Caco-2 > Dendritic cells > HepG2. These hierarchies are an outcome of the dose-response curves, where the response is an average of viability determined by 2-3 different assay methods (MTT, NR and Alamar blue). It should be pointed out that when forming a sensitivity hierarchy based on EC_{50} data, a different pattern emerges. This difference is attributed to the different functional dependence of viability on concentration observed for the different cell lines. Therefore, the choice of the cut-off for toxicity may influence the observed hierarchy. Moreover, the modeling enables to assess the influence of exposure duration on the toxicological outcome. The comparison of the cytotoxic effects induced by Co-NPs aggregates with their respective Co-ions which leached into the medium shows higher toxicity for Co-NPs when using the Caco-2 cell model at concentrations ≤100μM both for 48h and 72 h duration. At the same concentration, A549 and NCIH441 lung cell models show higher toxicity for the Co-NPs only for the 48h exposure. The dendritic cells showed a higher toxicity for Co-NPs at 72h at concentrations 50μM < C ≤ 100μM and. In addition, at concentrations ≤ 200μM the NPs were more toxic than Co-ions for both exposure durations.

The toxicological effects of Co-Fe ($CoFe_2O_4$) NPs were examined using seven different cell lines representing lung (A549 and NCIH441 cell lines), liver (HepG2 cell line), kidney (MDCK cell line), intestine (Caco-2/TC7 cell line), and immune system cells (primary mouse dendritic cells and a human B-lymphocyte cell line (TK6)). In addition, rat precision cut lung slices were examined. Dose-response curves were carried out in the concentration range of

0.05 -1.2 mM, employing MTT, neutral red and Alamar blue as viability endpoint assays following exposures of 24 and 72 h.

Data analysis and modelling of the obtained data sets was based on the decision tree model learned from the consolidated results after applying the KDD process. The concentration of the Co-Fe NPs emerged to be the most informative (first rank) parameter for toxicity prediction. The cell type turned out to be the second rank parameter. The third and the lowest rank in the model was either the time of exposure or concentration depending on the cell type. This model suggests the following hierarchy of cell sensitivity towards the toxicological insult of Co-Fe NPs: TK6 > Lung slices > NCIH441 > Caco-2 = MDCK > A549 > HepG2 = Dentritic cells, where the two cellular models of the immune system consisting of B-lymphocytes (TK6) and primary dendritic cells turned out to possess the highest and the lowest sensitivity, respectively

Mechanistic aspects of interaction, uptake and recycling of selected NPs by the different cellular systems

We have selected quantum dots as the prime NP type and gold NPs as a secondary NP type for uptake studies and intracellular recycling studies. Studies based on radioactive labeled NPs was carried out employing gold and Co-Fe NPs. Optical monitoring by confocal microscopy of NP uptake was pursuit using carboxylated, amine and pegilated QDs, based on the far red emission of the NPs, we have that Quantum Dots, especially the carboxyl-QDs and not the other two QDs have been observed internalized in all cell types examined, additionaly, preliminary inhibitors experiments have shown that the carboxyl-QDs uptake is probably through Macropinocytosis and Phagocytosis pathway but can also connected to clathrin-dependent endocytosis, experiments are being conducted these days in TAU and INSERM labs . The optical monitoring of gold NP uptake was demonstrated in dendritic cells, using reflection microscopy. The intracellular localization in membrane-bound vesicles of cobalt ferrite and gold NPs was also visualized by electron microscopy in alveolar cell types. Using radioactive labeled and unlabeled gold NPs, the uptake and the intracellular distribution of NPs in MDCK and HepG2 cells was investigated. Finally, it can be concluded that nanoparticles are taken up by different cellular systems and their internalization depends on the properties of the particles, the particle chemistry, and the length of exposure to NPs and on the different cellular systems used**.**

Exploration of toxicity mechanisms emerging following the interaction of NPs with the different *in-vitro* cell models

Experiments on the oxidative stress induced by cobalt ferrite NPs **i**n Caco-2 demonstrate that they possess ROS generating potential, being able to decrease glutathione level (an important anti-oxidant of the cell) and to increase intracellular ROS measured by flow cytometry using dichlorodihydrofluorescein as an optical probe. Similar studies of ROS are being conducted using the other types of cell models. The possibility of NP- induced programmed cell death was initially examined in HepG2 and MDCK cells following their exposure to cobalt ferrite however, no significant apoptotic processes could be detected. No inflammatory effects were induced in rat precision cut lung slices, HepG2 and MDCK cells (IL-8 released in the cells supernatants) in response to gold NPs and cobalt ferrite, however, it was shown that IL-8 secretion is slightly increased following incubation with cobalt ferrite NPs in Caco-2 cells and is parallel to the toxicity and the oxidative stress results**.** it was found that P601 and P703 do not induce any genotoxicity and morphological transformation effects. Exploring genotoxicity and genomic instability in lymphoblastic cells (TK6) and Balb/3T3 fibroblasts found no genotoxicity and/or genomic instability in the cells, also the concentrations were sub-toxic.

Exploration of NP-induced metabolic changes using precision-cut liver, kidney and lung slices by metabonomics

In an attempt to elucidate mechanistic aspects underlying the interactions of NPs with organ

tissues, we chose to elucidate their effects on the metabolic pathways of lactate, glutamine and glucose studied by a cellular metabolomic approach. This approach combines the incubation of precision-cut rat liver, kidney-cortex and lung slices with ^{13}C-labelled lactate, glutamine and glucose, with the enzymatic and carbon NMR measurements of substrate removal and product formation. The results of the latter measurements have been combined with mathematical models of lactate, glutamine and glucose metabolism that have been developed and validated in-house for each tissue used. The results obtained provide a panoramic view of the metabolism of each of these substrates and of the interactions of nanoparticles with the pathways involved. Lactate, glutamine and glucose metabolism has been studied in both the liver and kidney in the absence and the presence of the nanoparticles of interest. For the lung, only glucose appeared to be metabolized at significant rates. The results provide absolute values for fluxes through the key-enzymes of lactate and glutamine metabolism in the rat liver and kidney and of glucose metabolism in the liver, the kidney and the lung.

Only the effect of cobalt containing nanoparticles has been studied on the basis of their effects observed with enzymatic measurements. Indeed, the other nanoparticles tested did not alter the metabolism of lactate, glutamine or glucose in the rat liver, kidney-cortex or lung. Since their effects may be due to the leakage of cobalt ions, the effect of these has also been studied by cellular metabolomics. The overall results indicate that:

(1) The adverse interactions of cobalt-containing nanoparticles with the pathways of lactate, glutamine and glucose metabolism are tissue-specific and can be explained only partially by the leakage of cobalt chloride,

(2) The cellular metabolomic approach employed is liable to identify these adverse effects and the enzymatic pathways and steps that are affected by these nanoparticles,

NP-induced activation and inflammatory response of the immune system

Experiments performed in the presence of carbon nanotubes (standard multiwall nanotubes of 20-50µm) indicate that for the tested concentrations there is no cytotoxicity and no activation of dendritic cells (DC). Tested QD were shown to be highly cytotoxic: they induced the death of DC, but did not activate DC since DC bearing a high amount of MHC class II molecules did not vary upon incubation in the presence of these NPs. The influence of the NPs on the capacity of DC to secrete cytokines was tested after stimulation in the presence of various inductors. It was shown that most of the NPs, when used at high concentrations, reduced the secretions of IL12, but this activity could be restored to its normal level if the strength of activation was increased. A pre incubation of human serum with the different NPs tested in the CellNanoTox programme did not induce complement activation. These results indicated that even if the NPs had no apparent effect on the viability or on the phenotype of the cells, they may perturb their functionality.

Analysis of the database obtained throughout the project by Knowledge Discovery from Data (KDD) and Data Mining (DM)

A central activity of the consortium was to introduce the domain of Knowledge Discovery from Data (KDD) and Data Mining (DM) for integration and modeling of the obtained results. The KDD methodology was applied to the toxicity of Co-ions and Co-NPs in the different cellular models. The KDD goal was to discover rules for determining the toxicity of nanoparticles from the experimental results. The input data set included the consolidated experimental results, where each data record has the following attributes: (1) Cell type; (2) Particle type (Co-ions or Co-NPs); (3) Concentration; (4) Exposure time; and (5) The extent of viability decrease. Using a decision tree model, it was established that concentration is the highest rank parameter. The second rank parameter emerged to be either the compound type (Co-ions or Co-NPs) or the cell model, depending on the concentration range. The third and the lowest rank in the model was exposure duration. Furthermore, direct effects of the aggregated NPs on cells were observed in addition to their indirect effect due to the dissolution of cobalt ions from Co-NPs.

CellNanoTox Partners

Coordinator:

Tel Aviv University
Department of Physiology and Pharmacology of the Faculty of Medicine

Tel Aviv, Israel
Prof. Dr. Rafi Korenstein

www.fp6-cellnanotox.net

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

Department of Industrial Engineering of the Faculty of Engineering
Tel Aviv, Israel
Prof. Dr. Oded Maimon

→ Data Mining and Knowledge Discovery

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

JRC

Joint Research Centre
Institute for Health and Consumer Protection (IHCP), Ispra, Italy
Dr. Francois Rossi & Dr. Jessica Ponti

→ In-vitro toxicology, NP synthesis and characterization by physics-chemical methods

Westfälische Wilhelms University of Muenster

Institute of Mineralogy
Muenster, Germany
Dr. Ute Golla-Schindler

→ NPs analysis by electron microscopy and mass spectroscopy

Institute of Pathology
Mainz, Germany
Prof. Dr. C. J. Kirkpatrick & Dr. Ronald Unger

Johannes Gutenberg University of Mainz & Universitätsmedizin der Johannes Gutenberg University of Mainz

→ In-vitro study on NPs-cell/biomaterial interaction, effect on alveolar and endothelial cells

BASF SE
Experimental Toxicology and Ecology
Ludwigshafen, Germany
Dr. Robert Landsiedel & Dr. Susanne Kolle

BASF SE

→ NPs interaction with lung

CERICOL
Vinci, Italy
Dr. Giovanni Baldi

Colorobbia Italy Spa

→ NPs synthesis, functionalisation, modification

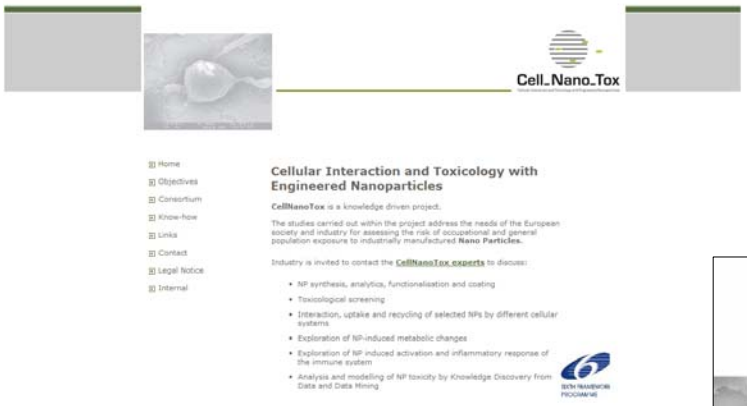
Project Management
Saarbruecken, Germany
Dr. Petra Zalud & Dr. Hanno Wittig

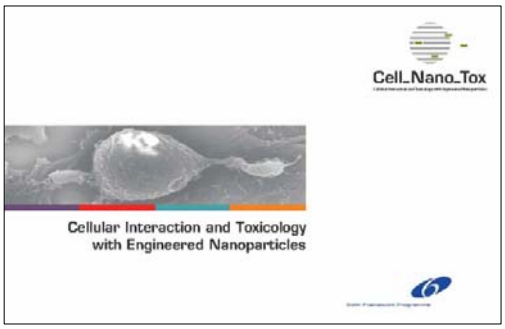
tp21 GmbH

→ Project Management, Public Relations

Dissemination and use

Over the lifespan of the project, the partners have communicated the scientific work and the goal in the frame of conference contributions, poster presentations, talks at conferences, networking participations. Finally 13 peer-reviewed publications have been released. The public CellNanoTox website links to all FP6 and FP7 nano material related projects and initiatives; it presents major information on nano material used and the related knowledge gained. The CellNanoTox website is a contact platform for industry to find experts for various assay methods to assess Nano-Bio interaction or NP know-how and will be available beyond the project duration. For awareness the consortium released a project flyer that has been distributed at all suitable opportunities such as conferences, networking meetings, universities. The flyer is still available and can be ordered via the website beyond the project duration. The flyer is a first basic presentation of CellNanoTox goal and scientific approach.





CellNanoTox partners are involved in several nano-particle related European and international projects such as NanoSafe 2 and NanoCare. CellNanoTox could harmonize protocols on nano particle handling with NanoSafe 2 and NanoCare. The partner BASF is member of all the projects and ensures the contacts between the coordinators. In addition, all partners are members of the European Network of Excellence Nano2Life. CellNanoTox partners participated in the N2L scientific meeting 2007 of the network and discussed scientific questions and results related to engineered nano-particles. Partners discussed future joint nano-particle related project applications. Being a member in the NanoSafety Cluster, the consortium had a strong scientific exchange with many NP related consortia. The project is presented in the 'Compendium of Projects in the European NanoSafety Cluster**.**

On the CellNanoTox web page, the consortium offers to be contacted by industry and stakeholders via its know-how page. A list of nanomaterials used and first recommendations are presented.

Publishable results of the final plan for using and disseminating the knowledge:

CellNanoTox's achievements and exploitable results in the **three knowledge areas** have industrial potential for commercial application or for developing, creating or marketing a product or process or for creating or providing a service:

1. 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. CellNanoTox developed expertise and experience in the production, analysis and the handling of engineered nano particles, and in production of NPs' dispersions in biocompatible solvents (glycols) of non-aggregated organic coated and uncoated NPs and in the synthesis of fluorescent or radiolabeled NPs without affecting the particles' chemical and other properties. After the project end **experts for NP synthesis – analysis and labelling to be contacted by interested industry to discuss individual NP needs.**

Exploitable Result: Nanoparticle synthesis know-how

Exploitation potential: New multifunctional nanomaterials with more than one chemical/physical function at the same time, making them compatible with widely differing applications in a range of fields, including glass, ceramics, textiles, biomedical, pharmaceuticals, building, agro-food and cultural heritage conservation.

Potential customers: NP-producing industry, Manufacturers from various industrial fields

Use after project end: Colorobbia Italy can be contacted to discuss the possibility to licence nanomaterial synthesis know how.

2. Nano-Bio-Interaction

Exploration of NP-induced metabolic changes using precision-cut liver, kidney and lung slices by metabonomics

The metabonomic approach in tissue slices using carbon 13 NMR and mathematical modeling of metabolic pathways has been carried out. This approach combines the incubation of precision-cut rat liver, kidney-cortex and lung slices with ^{13}C-labelled lactate, glutamine and glucose, with the enzymatic and carbon NMR measurements of substrate removal and product formation. The results of the latter measurements have been combined with mathematical models of lactate, glutamine and glucose metabolism that have been developed and validated in-house for each tissue used. The results obtained provide a panoramic view of the metabolism of each of these substrates and of the interactions of nanoparticles with the pathways involved. Lactate, glutamine and glucose metabolism has been studied in both the liver and kidney in the absence and the presence of the nanoparticles of interest. For the lung, only glucose appeared to be metabolized at significant rates. The results provide absolute values for fluxes through the key-enzymes of lactate and glutamine metabolism in the rat liver and kidney and of glucose metabolism in the liver, the kidney and the lung. The overall results indicate that: (1) The adverse interactions of cobalt-

containing nanoparticles with the pathways of lactate, glutamine and glucose metabolism are tissue-specific and can be explained only partially by the leakage of cobalt chloride; (2) The cellular metabolomic approach employed is liable to identify these adverse effects and the enzymatic pathways and steps that are affected by these nanoparticles.

Exploitable result: Contract service based on cellular metabolomics

Exploitation potential: Service for cellular metabolomics applied to the prediction of the safety and efficacy of test compounds (drug candidates, biologics, chemicals, cosmetic and agrofood products). To offer this service, the spin-off Metabolys has been founded.

Metabolys is a company with currently 8 employees, founded in November 2008 in Lyon (F) at the Laennec Faculty of Medicine (Lyon 8) by Gabriel Baverel (CellNanoTox member), University Professor and Hospital Practitioner, former Director of the INSERM Research unit 820 (Metabolomics and Metabolic Diseases) and Head of the Department of Renal Function Exploration at the Edouard Herriot Hospital. Metabolys is a spin-off from the Claude Bernard-Lyon 1 University and the INSERM.

Potential customers: Clinicians, NP-producing industry, Pharmaceutical industry, Researcher

Use after project end: Metabolys has 2 complementary sectors of activity: on the one hand, it offers contract services for various industries e.g. cellular metabolomics applied to the prediction of the safety and efficacy of test compounds (drug candidates, biologics, chemicals, cosmetic and agrofood products) and, on the other hand, it has the ambition to discover new antidiabetics at the preclinical development stage. Proof of concept for the use of novel NPs.

NP-induced activation and inflammatory response of the immune system

Experiments performed in the presence of carbon nanotubes (standard multiwall nanotubes of 20-50µm) indicate that for the tested concentrations there is no cytotoxicity and no activation of dendritic cells (DC). Tested QD were shown to be highly cytotoxic: they induced the death of DC, but did not activate DC since DC bearing a high amount of MHC class II molecules did not vary upon incubation in the presence of these NPs.
The influence of the NPs on the capacity of DC to secrete cytokines was tested after stimulation in the presence of various inductors. It was shown that most of the NPs, when used at high concentrations, reduced the secretions of IL12, but this activity could be restored to its normal level if the strength of activation was increased.

Exploitable result: Inflammation assay

Exploitation potential: Inflammation assay for risk assessment for industry in the frame of NP production

See also: ***Analysis of the toxicity of gold nanoparticles on the immune system: effect on dendritic cell functions.***
Villiers C., Freitas H., Couderc R., Villiers M.-B., Marche P.
Journal of Nanoparticle Research **12**, 1 (2010) 55-60
http://www.hal.inserm.fr/inserm-00458282/en/

Potential customers: Clinicians, NP-producing industry, Pharmaceutical industry, scientists

Use after project end: Appropriate strategies to develop improved biocompatible therapeutic and diagnostic agents e.g. for cancer therapy leading to shortening the overall validation process and limiting unsuccessful projects.

3. Knowledge Discovery

In CellNanoTox KDD has been used to support the investigation on toxicological effects of engineered NPs. The set up of a database has been started and will further elaborated made available to the stakeholders beyond the project duration.

The main benefit of KDD is that the abundance of data and variety of attributes generated in research can be translated into a meaningful causality compact model that explains the topological phenomena, and points out the order of importance of the attributes on toxicology.

The level of complexity reduces from the most complete data base for the scientific community to that one to be employed by the general public. This will serve as guidelines for new possible production processes and protocols on standardisation of toxicity assays. CellNanoTox RTD results will support future recommendations and norms.

Analysis of the database obtained throughout the project by Knowledge Discovery from Data (KDD) and Data Mining (DM)
A central activity of the consortium was to introduce the domain of Knowledge Discovery from Data (KDD) and Data Mining (DM) for integration and modelling of the obtained results. Parts of this activity were demonstrated in the analysis and modelling of toxicity induced by Co-NPs, Co-ions and Co-Fe NPs.

Exploitable result: Data Mining and Knowledge Discovery Know-how

Exploitation potential: Lay down the basis for analysis of large data bases on NP toxicology in order to examine the relationship between the physicochemical properties of NPs, dose response, exposure duration and cell models. An additional outcome was the creation of a broad critical and commented database on the health, safety and environmental impact of nanoparticles, which started in the frame of the FP7 project NHECD.

Potential customers: Researcher, Standardisation authorities, industry

Use after project end: On long term view, one major outcome for sustainable use is the Data Mining Know-how to support a better understanding of the impact of engineered nanoparticles on health and the environment and probably to discover previously unknown patterns. KDD know-how is available at CellNanoTox partner TAU.

A first info guide for industry on nano material used is available on the CelNanoTox website

NPs used	Knowledge gained, assay methods used, impact for industry in future
Commercial cobalt Nps	large aggregates, major ion leaching, toxic
Commercial carbon nanotubes	large aggregates, interfere with the toxicological methods
Gold NPs	Internalized the cells, nontoxic
Radioactive Gold NPs	Can replace EM research
Cobalt Ferrite NPs	Better magnetic properties, sub-toxic
Radioactive cobalt ferrite NPs	Can replace EM research
Fluorescent coated cobalt ferrite NPs	Less toxic than the naked form, can mark intercellular compartments
Quantum dots (JRC)	Can contribute to cell-nanoparticles interaction research
Commercial Quantum dots (3 types with different chemical surfaces from Invitrogen)	Amino and non-targted QDs can mark intracellular space and carboxyl-QDs can mark intercellular compartments

CellNanoTox peer-reviewed publications:

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   Bovine Serum Albumin-Based Magnetic Nanocarrier for MRI Diagnosis and Hyperthermic Therapy: A Potential Theranostic Approach Against Cancer
   Small, 2010, 6 (3), 366-370.

2. G. Baldi, G. Lorenzi, C. Ravagli,
   Hyperthermic effect of magnetic nanoparticles under electromagnetic field
   Processing and Application of Ceramics, 3 [1-2] (2009) 103-109.

3. Chiara Uboldi, Daniele Bonacchi, Giada Lorenzi, M. Iris Hermanns, Christine Pohl, Giovanni Baldi, Ronald E. Unger and C. James Kirkpatrick.
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