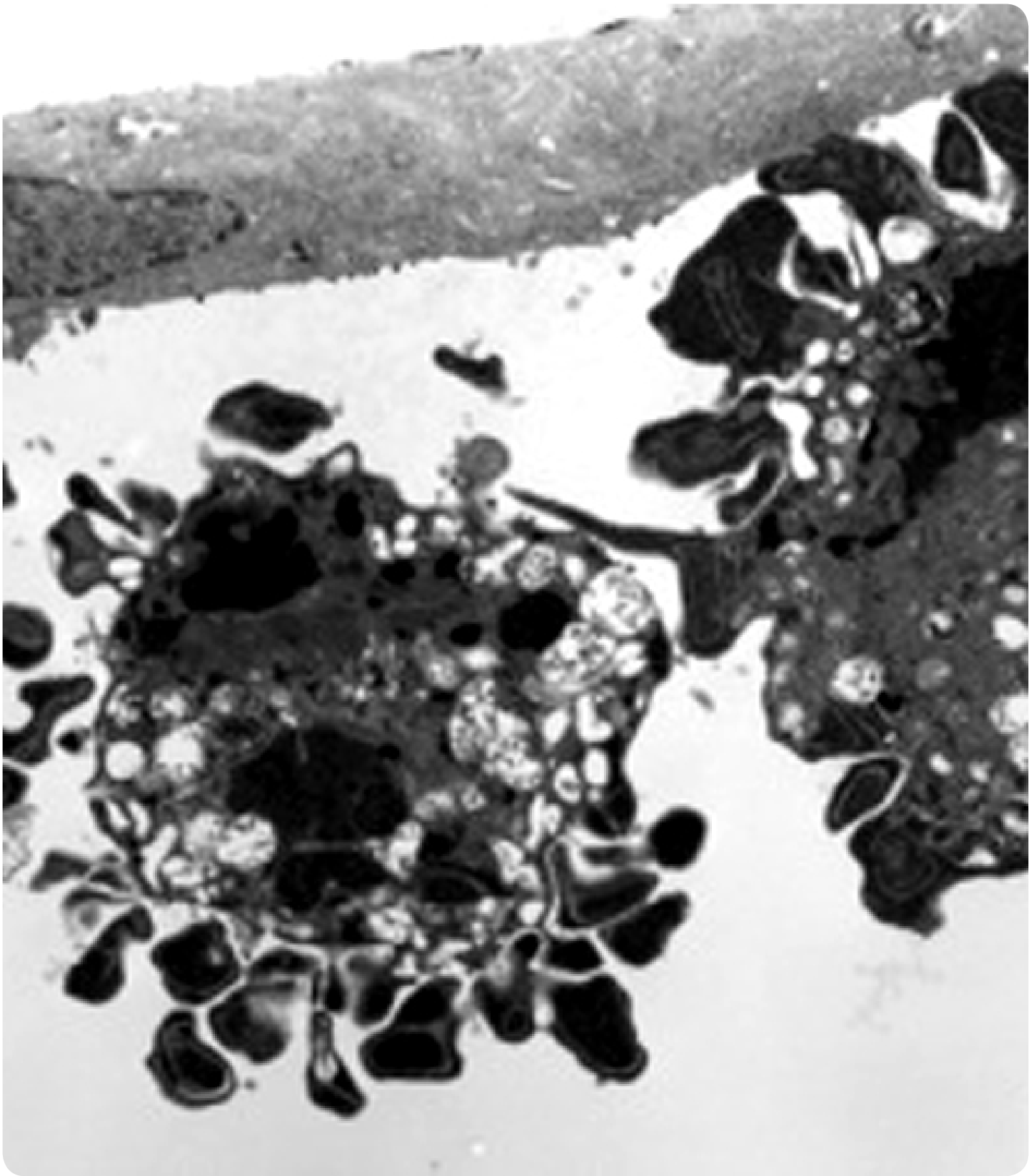
NanoDiaRA

Particles, Molecules & Cells · Diagnosis in-vitro & in-vivo · Rheumatoid Arthritis & Osteoarthritis

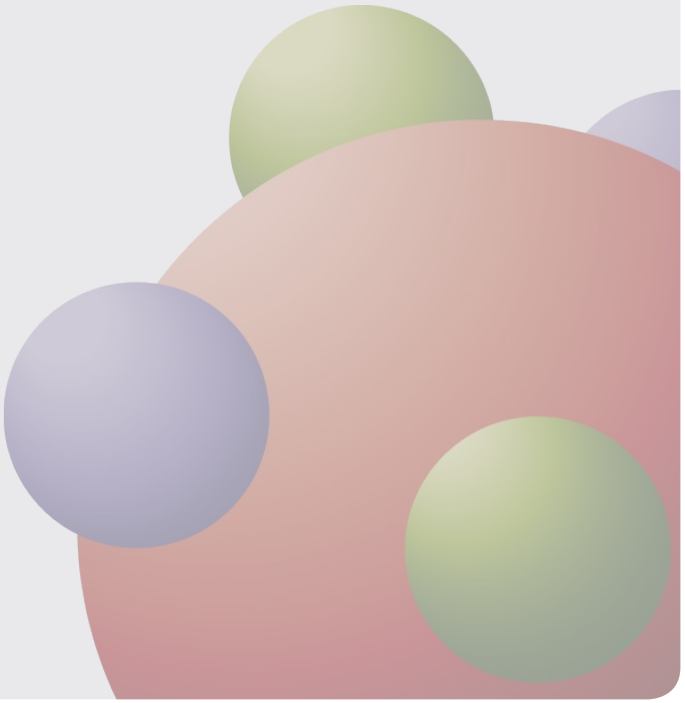
May 23, 2012

Nanoparticles in Medicine: Toxicity Methods and Standards

Summary and recommendations from an expert meeting and a discussion at the **École Polytechnique Fédérale de Lausanne, EPFL, Switzerland**



Contents



Frontpage: HeLa cells incubated for 24h with PVA-SPIONs MB1 0.8mg/mL.

Introduction

Inorganic nanoparticles evolve more and more in medical applications – in diagnosis and therapy for delivering drugs and in other biological and non-biological substances to specific types of tissues or cells. They have unique size-dependent properties and are therefore of high interest in many areas. Being smaller than around 100 nm, the particles are tailored and functionalized for being able to attach to, or enter in, diseased cells, and may even cross barriers like cell membranes and tissue barriers such as, for example, the air-blood-barrier in the lung or the blood-brain-barrier and many other internal tissue barriers. These properties are used for contrast agents as magnet resonance imaging or for targeted therapy using, e.g., highly toxic cancer therapeutic drugs to enter tumour cells. Both applications may improve the way of diagnosing and treating diseases. Being used in medicine, nanoparticle research and development is part of *nanomedicine,* defined to *"significantly change medical events only elucidated by concentrating on nanoscale events"*[1].

As nanoparticles are of peculiar interest for their application in biology and medicine and commercial exploration has become a topic, the translational research has to take into account that methods for characterizing the nanoparticles along the whole value chain from synthesis to the final product should be available to (i) define the particles by their properties and (ii) to predict their behaviour in the human body not only at the point of time of contact but also for several years after, so that even long-term side effects can be predominantly excluded.

The influence on the cellular machinery and the understanding of biological processes on the nanoscale level are necessary for using characterisation methods and tools to discriminate between toxic and non-toxic behaviour of the nanoformulations and by this to decide for or against a marketable product.

Aim of the workshop

Although scientists and clinicians have been active in the field of nanomaterials and – more specific – in that of nanoparticles for more than 20 years, the development of methods and standards required for testing especially inorganic nanoparticles and their impact on human cells and tissues is still ongoing and has not been satisfying yet. Tests measuring the influence of particle size, form, charge and protein absorption to the surrounding tissue, the influence of the uptake by the lymphatic or the blood system etc., are not standardized and may be challenging for the translation of research to marketable products for medical application.

The NanoDiaRA workshop ***"Nanoparticles in Medicine: Toxicity Methods and Standards"*** assembled experts dealing with nanoparticles starting from their synthesis processes and covering the whole characterisation chain from pure physical testing to investigations with the human interface. The organisers aimed to discuss with experts the current unsatisfactory situation in academic research by using methods of nanoparticle characterisation *in vitro* and *in vivo* which are not always meaningful and rarely used in a standardized manner. Too many variations in the investigations and the sometimes very vague descriptions of materials and methods prevent a scientific comparison of the published results. Furthermore, standards in industry are not yet implemented as legal guidelines. Presentations during the workshop highlighted the requirements from science and market and were followed by group discussions providing suggestions and recommendations.

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1 T. J. Webster. Nanomedicine: what's in a definition?, Int J Nanomedicine 2006 June; 1(2): 115–116. .

Presentations

With its presentations the workshop referred to different topics and questions in regard to nanoparticles and their impact on the human by presentations of Peter Gehr, University of Bern, Heinrich Hofmann, Ecole Polytechnique Fédérale de Lausanne (EPFL), and Yuri Volkov, Trinity College Dublin, and informed about the strategies of nanosafety research and nanosafety strategies and testing methods by presentations of Albert Duschl, University of Salzburg, and Peter Wick, Swiss Federal Laboratories for Materials Testing, Empa.

In this talk *"How nanomaterials can enter the human organism; example lung"* ***Peter Gehr*** underlined that especially those nanoparticles of sizes between 1–100 nm may penetrate tissues and cells easier than the larger particles. Some of them may enter cells passively by physical interaction with the cell membrane. The human has different opportunities to get into contact with the nanoparticles, for example by the skin or by ingestion into the gastro-intestinal tract, but the easiest way is by inhalation into the lungs. Those nanoparticles that have been inhaled and deposited on the internal surface of the lungs can translocate in the lung periphery (alveoli of the gas exchange region) through the air-blood tissue barrier into the capillary blood. Via blood circulation nanoparticles can translocate into other organs of the body, like into the liver, spleen, brain etc. Therefore the lung is the main portal of entry for particulate matter, particularly if it is of nanosize. The mechanisms of penetration and translocation as well as possible health effects are currently studied very intensely. However, at this time the complex interactions of nanoparticles presented to a physiological environment are not fully understood yet. The conformation of nanoparticles by size, shape and the high surface-to-volume-ratio, influenced in addition by their different organic or inorganic coatings, lead to selective absorption of a variety of biomolecules and the formation of a nanoparticle-protein corona which also depends on the entry point of the particles into the body and the status of the patient.

In this context ***Heinrich Hofmann*** asked if one should discuss *nanoparticles rather as molecules than as a kind of zero-dimensional hard substance*. He referred to Klaus Wittmaack, Helmholtz Zentrum München, who stated in his publication (ACS Nano, VOL. 5 ' NO. 5 ' 3766–3778 '201) that with very few exceptions, nanotoxicity studies implicitly involved the assumption that the techniques developed for risk assessment of hazardous chemical substances can be applied in unchanged form to explore cell response in nanoparticle-laden media. Furthermore, this misleading approach has the consequence that the actual dose of exposure is ill-defined or, more often, completely unknown. Propositions such as using the surface of all nanoparticles in the cell media seem to lead to a better correlation between particle amount and toxicity effect, but still the number or surface which is in contact with the cells is unknown. Recently Hinterliter and Teeguarden (Particle and Fibre Toxicology 2010, 7:36)[2] developed an algorithm – based on known colloidal behaviour of fine particles – which calculates numerically the amount of particles touching the cell membrane of fixed cells in cell cultures. Applying these calculations, it was shown that the amount of particles touching the cell membrane strongly depends on the size of the particles and its agglomeration behaviour. Interpretation of results of investigations aiming to show the influence of particle size on toxicity is more than questionable, if these colloidal aspects are not taken into consideration. It is therefore important to get to know more about the colloidal behaviour of the different nanoparticles – often discussed in the same breath – and the calculation and experimental determination of the amount of particles in contact with cells to interpret the toxicity studies undertaken by many of the laboratories correctly.

Some new studies on various inorganic nanoparticles such as silica or carbon (as carbon nanotubes) were undertaken by the group of ***Yuri Volkov*** in Dublin who presented *Nanoparticle-induced protein citrullination: a pathogenetic link to autoimmune disease development*. Rapidly expanding manufacture and use of nanomaterials emphasize the requirements for thorough assessment of health outcomes associated with novel nanoparticle applications. Post-translational protein modifications catalyzed by $Ca2^{+}$-dependent peptidylarginine deiminases (PAD) have been previously shown to trigger immune responses including autoantibody generation, a hallmark of immune complexes deposition in rheumatoid arthritis. Volkov's group assessed whether diverse types of nanoparticles were able to promote protein citrullination *in vitro* and *in vivo*. The models of cultured human cells of various lineages exposed to silicon dioxide, carbon black or single-walled carbon nanotubes (SWCNT) were investigated in parallel with C57BL/6 mice exposed to respirable SWCNT. The reporter readouts included high-content cell screening for protein citrullination, PAD activity and identification of potential target post-translationally modified proteins. It was reported that nanoparticles are capable of inducing protein citrullination both in cultured human cells and in mouse lung tissues implementing a PAD-dependent mechanism. Cytokeratines 7, 8, 18 and plectins were identified as main intracellular citrullination targets. This is the first report demonstrating that the induction of protein citrullination in human cells and in mouse tissues, following the exposure to nanosized silica or carbon-derived nanomaterials, can directly contribute to the development of autoimmune diseases. Some of these points were also discussed by Bengt Fadeel in his publication in the SMW[3]. Related to findings of Volkov he claimed another mechanism based on the interaction of nanomaterials with macrophages which may impede the normal process of programmed cell clearance which was, how-

2 Hinderliter P.M., Minard K.R., Orr G, Chrisler W.C, Thrall B.D. Pounds J.G. Teeguarden, Particle and Fibre Toxicology 2010, vol 7, paper 36

3 B. Fadel, Swiss Med Wkly. 2012;142:w13609

ever, different for different particle formulations (Fadeel mentions that „mesoporous silica particles of different sizes does not affect subsequent macrophage engulfment of apoptotic cells, whereas pre-incubation of primary human macrophages with SWCNT negatively[4,5] affected uptake of apoptotic cells."). He further points out that afterwards the dead cells must be eliminated which may, among others, lead "to the suppression of pro-inflammatory cytokine secretion and the promotion of anti-inflammatory cytokine production and induces immunological tolerance". So it would be important to know if and how nanoparticles influence cell death or "if cytotoxic nanoparticles trigger immunogenic or tolerogenic cell death."

These thoughts already make clear how difficult it is to discuss *Problems and strategies in nanosafety testing*, the topic of ***Albert Duschl's*** talk. As nano-enabled products are by now part of our daily life, this leads to concerns about possible risks to workers, consumers and the environment. Suitable methods are therefore needed for routine monitoring at the workplace in nano-producing or nano-using industries. Products containing nanoparticles have to be made safe for consumers and also made safe for the environment post-consumption. Safety is also paramount when developing nanomaterials for diagnosis or therapy in medicine. Nanoparticles present some aspects which are equally relevant both for medical and non-medical use, thus, knowledge about safety assessment methods should be widely shared. Examples of nanoparticles presenting problems in safety testing (medical and non-medical) include, for example, interference with all light-based assays due to their particular nature, or adherence to plastic or glass. They may be actively taken up into phagocytic cells or be contaminated with biological and non-biological agents. Until today no company can make sure that there are no biologically/medically relevant variations in properties from batch-to-batch, which makes it difficult to determine safety by using only a few tests. Aging of nanoparticles occurs even ex vivo, but due to nearly instantaneous binding of biomolecules (proteins and others) upon contact with a biological system or an organism, nanoparticle development over time requires continuous monitoring of their properties. In addition, new generations of nanoparticles may show special interactions regarding the already discussed protein corona formation. These issues and the consideration of their relevance for different applications may highlight the needs to address novel strategies to establish robust, reliable and affordable safety testing of nanomaterials.

4 E. Witasp, N. Kupferschmidt, L. Bengtsson, K. Hultenby, C. Smedman, S. Paulie et al. Efficient internalization of mesoporous silica particles of different sizes by primary human macrophages without impairment of macrophage clearance of apoptotic or antibody-opsonized target cells. Toxicol Appl Pharmacol 2009; 239: 306–19.

5 E. Witasp, A. A. Shvedova, V. E. Kagan, B. Fadeel. Single-walled carbon nanotubes impair human macrophage engulfment of apoptotic cell corpses. Inhal Toxicol 2009; 21(Suppl 1): 131–6.

But *How safe is nanosafety research?* ***Peter Wick*** asked in his presentation, referring to thousands of publications in this field and the irreproducible number of particle formulations used in these studies. Although the new properties of engineered nanomaterials promise great expectations for industrial, scientific as well as medical application, the concerns which have been raised about their potential adverse effects that may result from the inevitable interactions between humans and the constituents of nanotechnology – the engineered nano-objects – have not been disposed yet for once and for all. As a consequence a new scientific discipline was created: nano-safety research. Its aim is to contribute to a safe development of nanotechnology and to analyse possible adverse effects on human beings or the environment as early as possible to avoid social and economic drawbacks. Even though it is obvious that products containing nanoparticles have to be as safe as any other product on the market, no testing strategy has yet been developed to approach this. On the contrary, it was shown that many methods including those recommended by the Organisation for Economic Co-operation and Development (OECD)[6] or International Organization for Standardization (ISO)[7] are not adapted to and not suitable for nano-objects. This might be the reason for contradictory and non-comparable results as currently seen for most of the tested nanomaterials. To overcome this challenge, the reliability and transferability of such tests, also with respect to regulatory demands, have to be optimized.

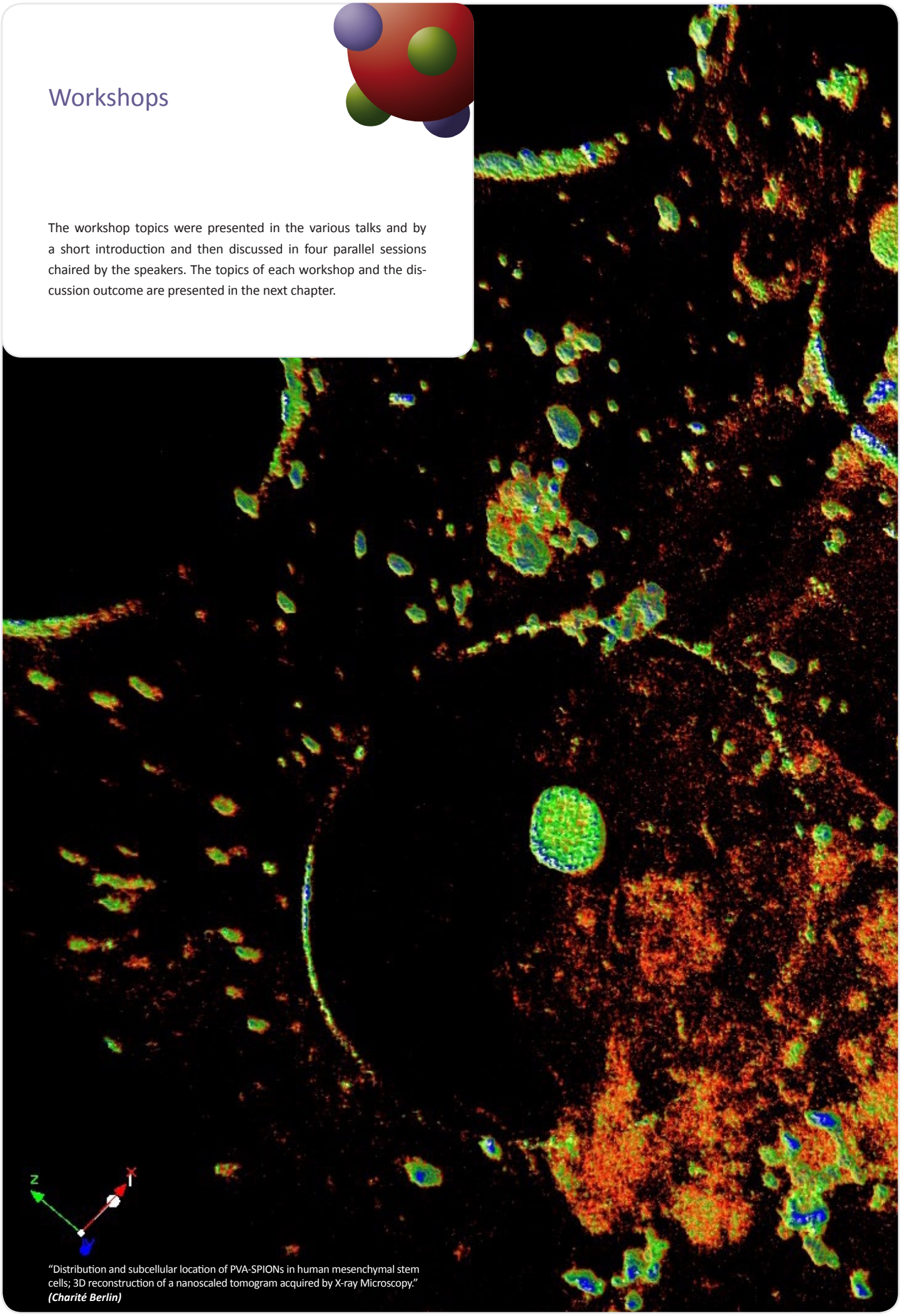
Therefore, the applied methods have to be validated carefully, a quality-management system has to be included and measurement of uncertainty as well as traceability of the whole system has to be implemented. In a joint action together with federal offices, industries, NGOs and research institutes EMPA started to harmonize and validate a set of *in vitro* methods addressing key aspects of cytotoxicity. To be able to provide a reliable testing strategy the detection and, if possible, avoidance of nanomaterial interferences as well as robustness are major goals of this activity.

6 http://www.oecd.org/science/nanosafety/44108334.pdf

7 ISO/TR 13014:2012, Nanotechnologies – Guidance on physicochemical characterization of engineered nanoscale materials for toxicologic assessment

Workshops

The workshop topics were presented in the various talks and by a short introduction and then discussed in four parallel sessions chaired by the speakers. The topics of each workshop and the discussion outcome are presented in the next chapter.



"Distribution and subcellular location of PVA-SPIONs in human mesenchymal stem cells; 3D reconstruction of a nanoscaled tomogram acquired by X-ray Microscopy."
(Charité Berlin)

WORKSHOP SESSION 1

Particle concentration in time and space

Chair: Heinrich Hofmann

Cellular cytotoxicity is dependent on the number of particles that interact with the cell culture per unit time and this is related to the sedimentation and diffusion rates of particles on the cell culture. The diffusion and sedimentation rates of the particles are dependent on their size and state of agglomeration, i.e., those particles with higher diffusion or sedimentation rates will on average be more frequently interacting with the cell. While the size of particles is relatively easy to determine experimentally and there exist models to predict the amount of particles coming into contact with cells, the agglomeration state of nanoparticles is rather difficult to measure. However, understanding the agglomeration behaviour of nanoparticles is important in order to predict the effect of particle size on in vitro particle-cell-interaction experiments.

The participants of this workshop focused on the question whether physical and chemical characterization of nanoparticles is sufficient to predict the interaction of particles with cell in in vitro and in vivo tests, for example, (i) type of concentration for cell test (mass/volume; surface/volume; surface/cell surface; particle number/cell surface and (ii) prediction about the change of particle characteristic during incubation (e.g., size, agglomeration, protein corona).

For nanoparticles used in medical application or in toxicity tests, a large number of literature exists containing long lists of parameters which have to be measured (see, for example, A. Nel et al.[8]). This characterization involves the particle granulometry, composition, morphology, the full characterization of the (bio-)fluids, the characterization of the behaviour of the particles in these fluids which comprises their interaction with the various biomolecules and, finally, the behaviour of the particles in the presence of and in contact with cells. At last, more than 20 different parameters have to be correlated with the outcome of standardized toxicity tests like cell viability (MTT,MTS, cell counting), oxidative stress, genotoxicity and immunotoxicity. Important to note is that these tests are developed and optimized for chemicals (molecules) and not for inorganic particles coated with polymers and covered by an eventually unknown number of proteins. What is measured in *in vitro* tests is, in the end, the response of the cells to the changes generated by the presence of nanoparticles in cell culture (examples: denaturation of proteins, adsorption of proteins and therefore changing of the composition, oxidative stress which alters the protein properties) but also the impact of the nanoparticles in the extracellular matrix or in the cytosol.

Most of the published results do contain not enough information about the experiments carried out and it is very difficult, in many cases even not possible, to compare the different results from different *in vitro* studies.

Therefore it is complicated to carry out meta-studies trying to establish more general relationships between particle properties and cell behaviour. A broad consensus between the participants existed about the loss of information due to fact that no basis exists for the proper presentation of materials and methods in most of the publications. It was therefore proposed to elaborate a check-list including all important experimental parameters to be communicated in a publication, as a supplement (if existing) or on a special web page. We like to refer to DaNa[9] as an example how to assess publication about toxicity studies or to give guidelines for future publications. Most of the nanoparticle in vitro tests today that use various cell populations do, however, not give sufficient information at all, as they do not deliver all necessary data.

In most of the cases it is not determined how many particles are in contact with the cells at a certain point of time or while the cell tests are performed. In their publications researchers do not communicate the amount of suspension per well and well size which makes the outcome of such tests highly questionable and the results not comparable with other investigations. Typical information like, for example, the concentration of nanoparticles in the cell media, are far from being enough to discriminate results from different laboratories, especially when evaluating the influence of the size of the nanoparticles on toxicity. The number of contacts of nanoparticles with cells in the medium is size- and time-depending (law of diffusion and segregation). The aim of further toxicity studies must therefore be to elaborate correlations between physical, chemical and biological parameters and to measure the response of cells. Thus a more mechanistic understanding of the particle can be established – cell interaction. To reach these goals it is necessary to further develop new *"Standard Operating Procedures"* (SOP) or use established ones for toxicity tests with nanoparticles and to publish them on a special "Nanoparticle SOPs Webpage". FP7 project *"Nanommune"* developed a "Quality Handbook"[10] with SOPs covering all different experimental protocols which were established by the partners of the projects, and which may provide "a useful manual for other academic or industrial investigators and small companies who are interested in safe and standardized procedures for nanomaterial synthesis and handling". However, regarding the unmet needs from research, one also has to consider an assessment of such SOPs regarding their significance on the particle type to be investigated and to assure that published SOPs are of high scientific level, good quality and informative value.

Beside cell tests performed *in vitro*, it is also necessary to investigate the characteristics of nanoparticles *in vivo* through animal models. This task covers the short- and long-term characteristics (toxicity) of

8 A. E. Nel, L. Mädler, D. Velegol, T. Xia, E. M. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson. Understanding biophysicochemical interactions at the nano-bio interface. Nature Materials 2009, 8(7): 583–57 and http://www1.cnsi.ucla.edu/institution/personnel?personnel_id=8739

9 http://www.nanoobjects.info/cms

10 http://www.nanosafetycluster.eu/uploads/QUALITY_HANDBOOK/NANOMMUNE_QHB_FINAL_2011.pdf

the particles. While the biokinetics can very well be performed with animal models, the long-term behaviour is much more difficult, as many of these particles cannot be tracked for very long times and it is difficult to assure that 100 % of the particles have left the body after a certain time. Drugs based on acetylsalicylic acid (Aspirin) or iso-butyl-propanoic-phenolic acid (Ibuprofen) have been available over the counter for decades now, but show long-term side effects which are more and more explored and have to be acknowledged. However, as in many other cases in medicine, the balance between benefit and harm has to be taken into account.

Handling the risks of nanoparticles at the best one has to raise the question if the existing in vitro tests are relevant in a first approach to define and restrict risks of toxicity – or are *in vivo* tests necessary in any way as living systems are different from what an *in vitro* test can implicate? Is it possible to work with simulated organs to reduce animal models? And if animals are necessary – which models are the most significant ones? The development of a series of "simple" tests, allowing early assessment of nanoparticles in development (see, for example, the VIGO-project for engineered nanoparticles, funded by the Swiss Competence Center for Materials) could be an interesting approach, at least for a selection of materials at an early stage of research.

Cytotoxicity

The effect of cell "vision" MUST be considered in interpretation of cytotoxicity data. In a biological fluid, proteins associate with nanoparticles and the amount and the presentation of the proteins on their surface could lead to a different *in vivo* response as uncoated particles would do.

However, in addition to protein adsorption, scientists should consider the concept of cell "vision" which is now recognized as another crucial matter that should be greatly considered for the safe design of any type of nanoparticles. The uptake and defence mechanism of cells, during interaction with exact the same amounts of nanoparticles, could be considerably different according to the cell types. Thus, what the cell "sees", when faced with nanoparticles, is most likely dependent on the cell type[11].

Nanoparticle Corona

While a remarkable progress has been achieved in understanding the hard corona composition, the current intercellular pathways nanoparticles undertake *in vivo* and their dependence on the corona composition have been neglected so far. Variation in plasma concentration can significantly change the fate of nanoparticles *in vivo* through alteration in the composition of the protein shell (*In vivo*, before reaching the cells, nanoparticles will be exposed to a variety of biological fluids which contain different protein compositions and concentrations; such nanoparticle pathways depend on the approaches by which the particles are administered into the body (e.g., subcutaneous, intradermal, intra-muscular, intravenous, intraosseous, intra-lumbar and by inhalation); thus, the evaluation of protein corona composition and its quantity, obtained from gradient complex media, are crucial in order to find out what the cell precisely "sees" *in vivo* when it interacts with the particles). This matter can help scientists to get a better understanding of the nanoparticle-cell interactions in vivo and elucidate the safety considerations for biomedical applications, resulting in naoparticles that are "safe by design".

Recommendations and further questions

It is recommended to develop tools which are able to detect the changes of cells in contact with the nanoparticles more sensitively, also knowing how many particles are in cell-contact and including signalling pathways. Without such sensitive tools it will always be difficult to identify the various interactions of nanoparticles in the human and their relevance to cells and, e.g., endocytotic pathways in the long term (ten years). VIGO may be a good example how to develop first screening tests (four different endpoints each with at least two methods providing a risk ranking).

The basis for the development of such tests and the reproducible outcome is the use of very well-defined nanoparticles. To allow the elaboration of meta-studies and therefore to make research in this field much more efficient, clear guidelines have to be written and agreed by the publisher regarding the information which has to be delivered within publications. A first example along these lines is the *DaNa* database.[12]

Limitations: It is possible to work with clearly defined particles for model experiments, however, in the real world and under normal production procedures particles show size and property distributions and are therefore not "defined" any more. As not enough systematic knowhow is available yet, it will not be possible to predict their behaviour. In addition, the transition from where a nano-behaviour may start as opposed to the bulk behaviour is not well-defined and will depend on each formulation individually. The EU definition of nanoparticles from 18.10.2011 – *"Nanomaterial" means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm–100 nm"* – does not help to solve the problem: It would need more precise declarations so that development can be carried out following a safe-by-design approach. It seems that size limit for physico-chemical properties is < 10 nm whereas for biological properties this limit is much larger than > 200nm.

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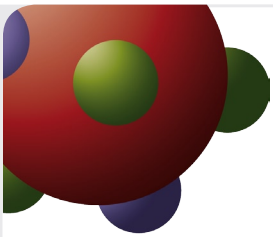
11 http://www.plosone.org/article/info:doi/10.1371/journal.pone.0029997 and http://pubs.acs.org/doi/abs/10.1021/nn2021088

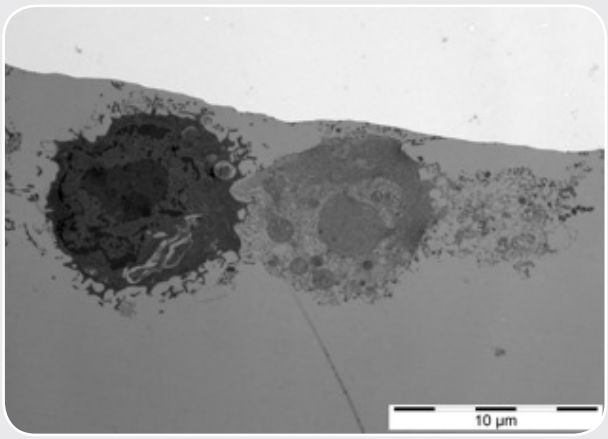
12 http://nanopartikel.info/cms/lang/en/Wissensbasis/kriterienkatalog



Additional comment from the Workgroup chair H. Hofmann:

The discussion as presented in the summary of Working Group 1 clearly shows that the existing methods of toxicity tests with inorganic nanoparticles still depict an unsolved problem. The huge amount of results is based on nanoparticles which are mostly insufficiently characterized or documented. The applied tests are very often taken from the arsenal of toxicity tests for molecules. It is very interesting to note that the on-going discussion in the United States regarding new approaches for toxicity tests for chemicals and drugs as well as the activities to reduce the amount of animal tests for cosmetics is not a subject in the community of nano-toxicology.

Approaches similar to system biology or computational system biology are missing (as an example see more sophisticated data analysis like fuzzy logic as it is used for the analysis of complicated systems). According to Bhattacharya[13] modern toxicity has to use approaches similar to system biology. For more details and discussions how to develop further toxicity evaluation, see also the publication of Andersen et al.[14] It is interesting to note that in the EU FP7 research programme, the last NMP call which will was published in July 2012 now addressed these topics (see. for example, NMP.2013.1.3-2: Nanomaterials safety assessment: Ontology, database(s) for modelling and risk assessment)[15].



RAW 264.7 cells incubated for 24h with PVA-SPIONs MB2 0.4mg/mL. ***(EPFL)***

Additional open questions regarding toxicity tests:

- Would particles have the same properties if they were in different organs and cells? Does the history of the pathway of the particle in the body have any influence?
- One problem of *in vivo* and *in vitro* studies with primary cells is that each individual has another "history of diseases" and therefore the results can be very different. This, though is a general problem concerning all biological studies and not only nano-relevant.
- A similar question could be asked for developing and growing an organism. Do nanoparticles have the same effect in an adult body as in a developing and growing body?
- Control experiments in *in vitro* to detect how many particles reach the cell surface are still not standard in toxicity tests.
- Influence of the porosity of the particles on transport and protein adsorption (silica particles or coatings with silica have a porosity of > 50 %).
- Determination of the ligand layer thickness
- Oxygen content during cell test?
- Influence of size distribution
- Injection rate can influence the aggregation rate of the particles (local high concentration).
- Can metabolism in the liver lead to toxic by-products?
- Are all parameters known which can influence the toxicity?
- Related to the previous question: Is this possible to get all the experimental values of nanoparticles in advance of the toxicity studies? Are all the experimental values really necessary? What could biologists do with them? Do we have enough knowledge to correlate correctly particle properties with toxicity data?

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13 S. Bhattacharya, Q. Zhang, P. L. Carmichael, K. Boekelheide, M. E. Andersen. Toxicity testing in the 21st century: defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. PLoS ONE 2011; 6(6); e20887.8.

14 M. E. Andersen, M. Al-Zoughool, M. Croteau, M. Westphal, D. Krewski. The future of toxicity testing. Journal of Toxicology and Environmental Health 2010; Part B: Critical Reviews, 13:214: 163–196.

15 http://cordis.europa.eu/fp7/cooperation/nanotechnology_en.html

WORKSHOP SESSION 2

Assessment of opportunities and risks of medicinal nanoparticles

Chair: Albert Duschl

Nanoparticles used in medical applications like diagnostics, drug delivery or therapeutic treatment are mostly exploiting their size-dependent properties, e.g., quantum size effects and the large surface area to volume ratio which make them unique compared to larger particles. They are used in "nanomedicine" to either increase the sensitivity or efficacy of diagnostic methods or therapies and, in parallel, to reduce possible side effects as, e.g., in case of cancer drugs. In comparison to the opportunities of nanomedicine, the exposure of humans to nanoparticle formulations and their possible toxicity is an important part of the scientific and social discussion. Most of the nanoparticle toxicity testing is based on in vitro methods and has been established either for normal pharmaceutical formulations or, in case of industrial nanoparticles, for hazard characterization of chemicals. Evidence is given that nanoparticles may interfere with commonly used test systems. Reliable toxicity test systems with standardized methods have not been fully developed yet and there is an urgent need to demonstrate the safety of nanomedicine. Still the following questions are not fully solved: (i) How can or shall nanoparticles enter the human body and how can or shall nanoparticles penetrate through tissue and cells? (ii) Which is the more important aspect for using nanoparticles: quantum size effects or the large surface area to volume ratio? (iii) Is the toxicity influenced by physicochemical properties or by the biological effect (signalling)?

While risk assessment of nanomaterials is of course a critical issue, it needs to be emphasized that these novel materials offer a range of *opportunities* not accessible to conventional chemical or biological agents. Some of the opportunities highlighted in the workshop session are indicated below.

Multifunctionality: Nanomaterials offer the possibility to combine multiple functionalities in a single entity. A single nanoparticle may thus be designed to, for example, accumulate in a specific organ, to target a specific tissue or cell type, to carry one or more drugs and to facilitate the penetration of defined barriers. This may be achieved by coating the surface with different agents or with sophisticated core-shell systems, which are now increasingly available.

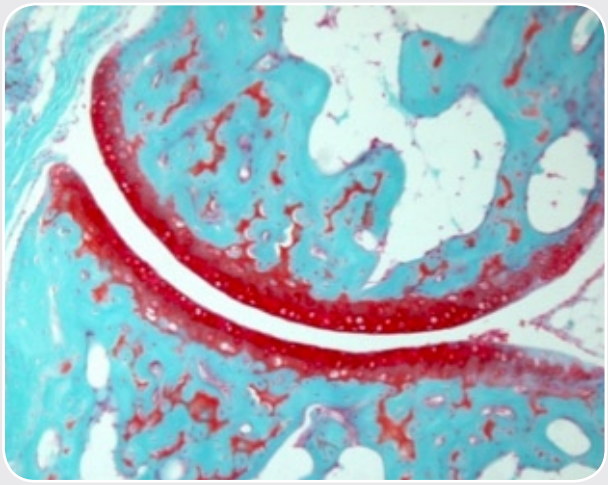
Targeting organs: It has been observed that some types of nanoparticles tend to accumulate in specific organs which are often, but not always, associated with mechanisms of excretion. Such a property can be used to target drugs to the defined organ. For example, a nanoparticle, which is excreted via urine, may be useful for treatment of bladder disease (e.g., cancer or bacterial infection). This can be enhanced by adding functions which prolong residence times, like addition of binding partners for markers of the selected organ. It is thus useful to observe the migration of nanoparticles in the body without any prejudice, since all organs are desired targets in some situations.

Targeting cells: Targeting of tumour cells is not easy since a tumour will often lack specific known markers on its surface. Some surface markers are successfully targeted, like HER2 in breast cancer, but targeting intracellular markers is more difficult. However, intracellular targets exist and are exploited, in particular by kinase inhibitors. Other targets may be highly tumour specific, like mutated $p21^{ras}$ protein. The potential of nanoparticles to enter cells may be exploited to deliver drugs intracellularly, in particular if this can be combined with targeting to a selected cell type. It can be envisioned that a nanoparticle targets a specific organ and delivers a medical agent that damages only tumour cells.

Penetrability: An issue of concern is the ability of nanoparticles to penetrate tissues and cell membranes. However, the same property makes them useful as drug carriers, so strategies to enhance the penetrating capacity could be useful. This refers both to penetration through barriers, like peripheral tumor tissue shielding tumor cells in the core, and to penetration of cell membranes, where significant mass transport is required to reach sufficient levels of a drug within the target cell. One option would be to mimic cell-penetrating peptide sequences from proteins like antennapedia or the HIV TAT protein.

Bacterial targets: While tumour cells often lack good markers the same is not true for bacteria, which provide bacterial patterns professionally recognized by immune receptors from the TLR, NLR, RLR and other families. The increasing resistance of some pathogenic bacteria to antibiotics makes it necessary to explore new approaches. Nanoparticles could be functionalized to recognize bacteria – based on the known immune receptors – and thus deliver bactericidal drugs at a high local concentration. Membrane-disturbing properties of particles could by themselves aid in the destruction of bacterial cells.

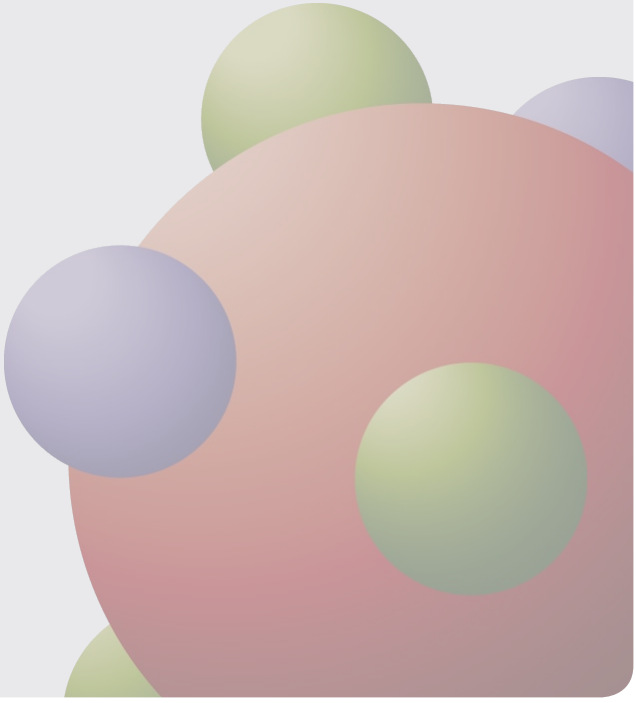
Development: Cost and time of development are the limiting factors for the drug pipeline. Nanosafety research can be useful by identifying problematic candidates early on, as this enables to quicker focus on more promising entities. If there is a need to stick with a specific particle type, safety research can identify the properties of the nanoparticle which are responsible for the toxic effect, like size, shape, coating, charge density etc. If it is known which property of a nanoparticle makes it toxic it may be feasible to re-engineer the particle for higher safety (achieving safety by design). Ideally it will be possible to modify only the one problematic parameter without losing the desired functionalities. In this case a molecular understanding of toxicity mechanisms may save a promising line of development, which would otherwise have to be abandoned.



Histological section of a healthy joint stained with Safranin O:
red surfaces indicate intact articular cartilage
(The Radboud University Nijmegen Medical Centre)

Persons at Risk: Safety research can enable to identify persons who are at risk of adverse reactions, like allergy or autoimmunity. Excluding such patients from clinical trials and later on from treatment would be highly beneficial.

Other options also exist, like development of personalized nanoparticle composition, cheaper production of drugs in this state or earlier diagnosis due to higher sensitivity. However, the examples given above already illustrate that nanoparticles hold very promising options for the development of novel drugs and that research into the associated risks and into mechanisms of toxicity has a high potential for supporting the development of nanomedicine.



WORKSHOP SESSION 3

From application to clearing:

I) - facts conceded as true and

II) - open key questions regarding the behaviour of nanoparticles

Chair: Peter Gehr & Yuri Volkov

Discussing in vitro assessment of the cell interaction with the nanoparticle gives a first indication of the behaviour of a nanoparticle in the living environment. Another question is whether such tests represent the in vivo behaviour of nanoparticles for the defined application and which in vivo tests can optimally mimick the activity of nanoparticles.

This all depends on the final application of the nanoparticles (as blood-pool contrast agent, as contrast agent for a targeted organ/tissue by using intravenous (iv) or intervenous (ia) as pathway, as drug delivery agent (oral, intravenous, nasal etc.), as therapeutic agent, e.g., in hyperthermia etc.) and the formulation of the nanoparticle as a physical particle with no further function or the pharmaceutical particle having biological or pharmaceutical ligands for specific targeting and therapeutic use. Questions which were discussed in this respect are: (i) How do nano-particles interact with biological membranes (e.g., air-blood barrier, blood-brain barrier etc.)?, (See also questions Workshop Session 2) (ii) Existing methods to detect the clearing behaviour of nanoparticles, (iii) Do inorganic nanoparticles have to clear the body?, (iv) Are the current methodologies for nanoparticle-interaction sufficient or is there a need for the development of new tools?

When discussing the interaction of nanoparticles with the human body one has to ask by which ways and how they can penetrate through tissue and cells. Nanoparticles interact with "biological membranes", e.g., air-blood barrier, blood-brain barrier, intracellular membranes, i.e., of organelles, nucleus etc., mostly consisting of double layers of phospholipids with glyco-proteins and glyco-lipids. Before a tissue membrane can be penetrated by nanoparticles, the nanoparticles have to enter individual cells or cells in a layer, i.e., they interact with a cell-membrane. Recent studies of Prof. Gerd Ulrich Nienhaus of the Karlsruhe Institute of Technology, KIT, showed that the physical/mechanical interaction of nanoparticles with the cell membrane cause the membrane fluidity to become more flexible, i.e., phospholipid molecules probably temporarily move apart and allow nanoparticles to slip through into the cell, into the cytoplasm (i.e. passively) where they are found free, i.e. not membrane-bound[16]. Prof. Nienhaus made calculations to these findings.

Another – and probably more frequent – way for nanoparticles to enter a cell is endocytosis (i.e., actively), probably by a caveolin mediated mechanism (Rothen-Rutishauser et al., submitted, 2012)[17]; these particles are membrane-bound. As far as the intracellular effect, trafficking etc., is concerned it is still questioned whether nanoparticles in the cytoplasm are free or membrane-bound. The most important question is then, how nanoparticles may enter the nucleus and interact with the DNA (or mitochondria and interact with the respiratory chain)? As this was unfortunately too time-consuming to be discussed at the Workshop, it should be taken into account when further discussing *in vivo* interaction of nanoparticles with cells.

Further points to consider are the quantum size effects and the large surface area to volume ratio of nanoparticles and the influence and importance of these effects on further biological effects, once these particles have entered the body (e.g. through signalling). In this respect, one has to be aware of the influence of the protein corona which is created around a nanoparticle after entering the body, the type of corona, its modification during nanoparticle penetration through body fluids, cells, tissue etc.

During the discussion it became clear that this field of cell-nanoparticle interaction is still at an infant stage and there still are numerous questions to be solved. Several examples were highlighted like, e.g., nanoparticle-cell contact when entering or leaving the cell. Not much is known, though, how and by what mechanism nanoparticles can leave the cell – will it be an active or passive interaction? It is a fact that they cross cellular layers as, e.g., during translocation from the air space into the capillary blood in the lungs (alveolar region). Dealing with the cell compartments more in detail, it is still an unanswered question how nanoparticles enter organelles (e.g., mitochondria, where they could interact with the structures of the respiratory chain) and the nucleus (interaction with the membranes or through the pores?). The next topic concerns barriers: There are many barriers for particles when entering the organism as far as nanoparticle penetration (translocation) is concerned, besides the air-blood tissue barrier (lung, skin), the lumen-blood tissue barrier (e.g., GI-tract), and these have to be further investigated. There are internal tissue barriers for particles which enter the blood stream to leave it again in any organ such as the blood-brain-barrier, the placenta barrier (circulation from mother to child), the blood-thymus barrier (maturation of T-lymphocytes), blood testis barrier (maturation of sperms) etc. Especially if nanoparticles are used as contrast agents for molecular imaging or for drug delivery, one or several of the mentioned barriers have to be overcome by the particles.

To answer such questions more easily one also has to concentrate on methods to detect the interaction of particles with barriers, cells, cell compartments and the clearing behaviour of nanoparticles. The process of clearance – whether this term should be used only for the case that particles quit the body or also if they move from one organ to another – depends on the type of particles and their location in the tissue and in the cell of a specific organ. This means that it should be defined wherefrom the particles might be cleared, from subcellular structures like e.g. nucleus, organelles, cytoplasm with other cellular structures like, e.g., the cytoskeleton, from cells, tissues, organs or even from the organism to make the right conclusions on the results received. Nanoparticles can be cleared via different pathways, having probably influence on the clearance mechanisms like, e.g., exhalation

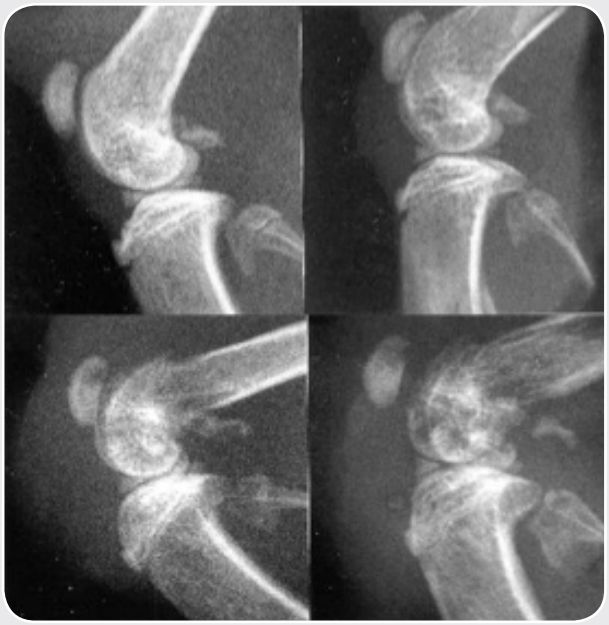
16 T. Wang, J. Bai, X. Jiang, G. U. Nienhaus. Cellular uptake of nanoparticles by membrane penetration: a study combining confocal microscopy with FTIR spectroelectrochemistry. ACS Nano 2012; 6(2): 1251–1259.

17 Petri-Fink, A, Rothen-Rutishauser, B; CHIMIA 66 (3)104-109

and mucociliary escalator (lung), feces (GI-tract), urin (kidney), via blood and/or lymph circulation. Using, for example, iron oxide as nanoparticle the additional term of transformation of iron to transferrin has to be taken into account; the storage of this iron in the blood pool will be another mechanism which can be observed and may influence the further clearance of the rest of the nanoparticles. One also has to discuss the clearance of the coating material, which – in most cases – is of synthetic or natural polymers.

Finally, one also has to discuss the fact that an inorganic nanoparticle may not leave the body but stays in cells or organs and accumulates. This question led to a lively and emotional discussion. Would this be dangerous or disturbing a health problem? Satisfying answers on dissolution or biodegradation (biopersistence) of nanoparticles cannot be given yet, as no methods are available to track single nanoparticles and their distribution in the body. Proposing the development of only biodegradable nanoparticles, particularly, e.g., in medicinal applications (nanomedicine: therapeutic, diagnostic), also has restrictions as only the inorganic nanoparticles have specific physical property which are needed such as the magnetism for contrast agent, surface plasmon or fluorescence for phototherapy etc.

Some recommendations were made regarding the ongoing research. Some important points should be considered regarding particles, dose, time and methods: (i) the nature/characteristics (physical, chemical), size, toxicity/pathogenicity, biodegradability, coating (effect of coating alone or coating+core) of particles should be defined and described in detail to interpret the results optimally; (ii) the dose/quantifications in combination with the size of nanoparticles would be of interest to better define the effects of doses. In addition, doses in cells or tissue should not exceed levels by which cells only die because of the quantity of nanoparticles and effects of their characteristics/functions are hidden. Similar the time effect: What role does time play, what is a chronic effect? It is still an unsolved problem to get comparison of *in vivo* and *in vitro*, in particular as far as dose and time are concerned. Barriers for nanoparticles may play a role and they should be defined and tested by different particle types (size, material, coating etc.).



Radiographic images of various stages of bone erosion in arthritic knee joints. Nanoparticles might provide useful additional diagnostic information, as contrast agent or as identifier of specific pathological processes.
(The Radboud University Nijmegen Medical Centre)

WORKSHOP SESSION 4

Are existing recommendations for toxicological evaluation of nanoparticle medicinal products sufficient?

Chair: Peter Wick

Regulation of nanoparticles for medical application have been discussed similar to those particles which are used for industrial reasons and the debate related to human health and safety risks associated with nanoparticles is similar for both, medical and industrial applications. In the last years the emotional tenor of debates has changed because of more scientifically based outcomes and various legal and scientific expert groups have made recommendations. Nevertheless, such non-legally binding proposals seem not to be sufficient while standards are not yet finalized to give standardized guidelines for companies and researchers to get answers on genotoxicity of nanoparticles – are nanoparticles cancerogenic? Or take immunotoxicity of nanoparticles: What happens with nanoparticles during and after they have done their job? The questions which were discussed in this session dealt with (i) the difference regarding synthesis characterisation, composition and administration between engineered nanoparticles and nanoparticles designed for medical application large enough to justify a different treatment, (ii) the distinguishing difference between medical device and pharmacotherapeutic in the use of nanoparticle and how to bridge it, and, finally, (iii) the need of a standardised approach for the safety assessment of nanoparticles from the regulatory standpoint.

Introduction

There are basically two types of approaches to the named problem: Bottom-up (science-driven) and top-down (regulation-driven), whereas the bottom-up approach works fine for the scientific point of view, but causes problems for the regulation side and vice versa. Due to the different requirements of the two sides, the two approaches do not really match: What is "safe" from a scientific point of view may not count in regards to regulatory aspects. Recommendations are existing, e.g., in a publication about risk assessment of engineered nanoparticles[18]. However, this report, like others, is a purely qualitative paper and does not always indicate which methodology to apply. There are still two basic questions to be answered:

- Are the existing regulations adequate for nanoparticles in the field of medical applications?
- What is missing for the regulators?

Common platform

Regulators expect the industry to offer a safety concept as well as concrete risk assessment studies of nanomaterials in their applications, whereas the industry expects being told what to do by the regulators (in particular nano-related issues). Therefore, a "waiting situation" has developed in which nobody gets active. It would be of great help to create a common platform as a "research hub", consisting of experts from very different fields (including, e.g., food) to ensure a broad approach. This would make sure that existing and new knowledge flows into the innovation chain. Dossiers and assays could be distributed at least partially to share the general workload. However, it is difficult to get people (especially from industries) to invest into such a platform, and, even more, to take the responsibility to lead it.

The industry will not invest into something that does not provide exclusivity in knowledge and has not developed yet. Interest will only develop if such a platform has reached a certain size as "knowledge pool". The Swiss "Competence Centre for Materials Science and Technology, CCMX"[19] is currently discussing an initiative to force the collaboration between academia and industry at a precompetitive level.

Definition of "Nano"

The definition of the term "nano" still is an important issue. Are there any objective measurements to judge whether something is nano or not? The purely size-dependent definition of 100 nm as a border does not always match, because there are entities below this size border (e.g., vesicles and other small bioorganic components) one would not count as nano. On the other hand, bigger particles (up to close to 300 nm) could be considered as nano-specific, because they are treated differently in the body as compared to their larger variants.

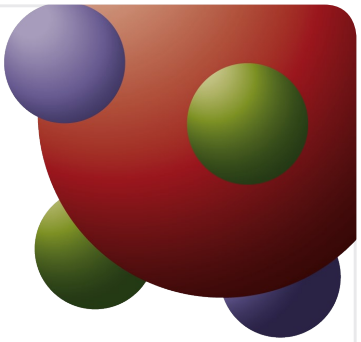
There are also compositional mixtures like, e.g., in titaniumdioxide (anatase, rutile) which are problematic when it comes to the correct nomenclature of the particles and thus lead to confusion about the identity of the investigated particle. As far as the interaction of nanoparticles with biological systems is concerned, there is evidence that nanoparticles larger than 100 nm "behave like so called nanoparticles (per definition)" which means that they enter, for example, red blood cells up to a size of <200 nm. It is suggested that one should think more in terms of "life cycles" and classifications should be made at different points of time, as "nano or not nano" can even change within a particle's lifetime. The questions whether one should establish property-specific differentiation criteria remains open for now. Other definition problems refer to question such as whether it depends on the origin of the component if something counts as "engineered" or not or the distinction between a drug or a medical device.

Quality checks

The German project *DaNa* defined a criteria catalogue to select papers that are accepted in their database. The criteria include correct controls, GLP conditions and adequate description of materials and methods. Materials and doses have to be justified to exclude, for example, overdose studies from the beginning. With tools like the Swiss Precautionary Matrix (as part of the Swiss

18 D. R. Hristozov, S. Gottardo, A. Critto, A. Marcomini. Risk assessment of engineered nanomaterials: a review of available data and approaches from a regulatory perspective. Nanotoxicology 2012; Jan 9.

19 http://www.ccmx.ch/about-ccmx/



Action Plan "Synthetic Nanomaterials")[20], a first check by the industry (after, or even before, basic development) is possible. It does not matter whether such a checklist (available online[21]) is complete or not, but it gives a first indication where knowledge might be missing.

In the platform mentioned above, a checklist for first tests could be established which gives a first orientation and serves as standard for mandatory data. After passing these basic checks, a further evaluation by platform members could follow. Only selected data in this context would be then used further and might eventually lead to adequate models. It has to be taken into account that there already might be implicit recommendations and that checklists in the form of official recommendations are a political "minefield".

Assessment of tests and models

It must be possible to derive information from tests even if, e.g., the particle size in another setting is not exactly the same. Therefore, it is necessary to move from purely descriptive to more predictive data. It is also suggested to evaluate risk-benefit ratio and life cycle assessment for each case. Very recently a handbook for standard procedures (Quality Handbook, Bengt Fadell, Krug (eds), Nanommune, 7th Framework Programme, 2012)[22] was published. Additional information is offered by FP7 NanoImpactNet (Research protocol list)[23]. As regulators are hardly interested in *in vitro* studies, especially the quality of *in vivo* models used for nanoparticle-cell interaction has to be validated. *Long-term, low-dose studies have to be conducted to study accumulative effects in the body.*

Recommendations will differ strongly depending on the field of application. The adequacy of a certain model can be judged scientifically only case by case. The state of the art is the delivery of a "normal" tox package which analyses the particles, the carrier and the combination.

Summary of the workshop and recommendations

The workshop covered questions on toxicity, safety, risk and legal issues over the lifecycle of a nanoparticle for medical applications from synthesis to elimination from the body. As nanoparticles may come into contact with the human body either by chance as natural or handmade particles or by products especially designed for the human application (e.g., for cosmetic purposes or even more pronounced) to enter the human body and to act for diagnostic purposes, as drugs or in any other therapeutic manner, one may define risks and chances differently. In the first two cases one may discuss only risks but has to balance between risks and chances for the human, whereas in the third case chances to cure a disease may sometimes be accompanied by possible risks.

20 http://www.bag.admin.ch/nanotechnologie/12167/index.html?lang=en
21 http://www.bag.admin.ch/nanotechnologie/12171/12174/index.html?lang=en
22 http://www.nanopartikel.info/files/content/dana/Dokumente/NEWS/NANOMMUNE_QHB_FINAL_2011.pdf
23 http://www.nanoimpactnet.eu/index.php?page=Researchprotocols

Regulation: Summary and Conclusions

- Regulators and industry are in a "waiting situation" concerning the toxicological evaluation of nanoparticle medicinal products, so the initiative has to be taken by others (the government or possibly the research community).
- There are no explicit checklists yet. Regular bodies will not define such a checklist, as this is a political "minefield". Therefore, the appliers have to develop such a list.
- The current concept of medical regulations is acceptable also for nanoparticles.
- Studies should be quality-controlled by defined criteria. They should focus on more predictive than descriptive data.
- It has to be answered whether the right models for the right questions are used. *In vivo* and in particulate *in vitro* models used for nanoparticle-cell interactions should be validated.
- The definition of "nano" in medical applications is unclear and should be discussed further (considering the fact that nanoparticles > 100 nm – maybe up to a few 100 nm – can easily enter cells).
- Dossiers and assays should be shared at least partially to share the general workload. It is foreseeable that with that, IP problems will become an issue.
- Make assays open, workload could be distributed. IP will then become the issue.
- It is easier to define what should be done than how to do it or even who could do it.

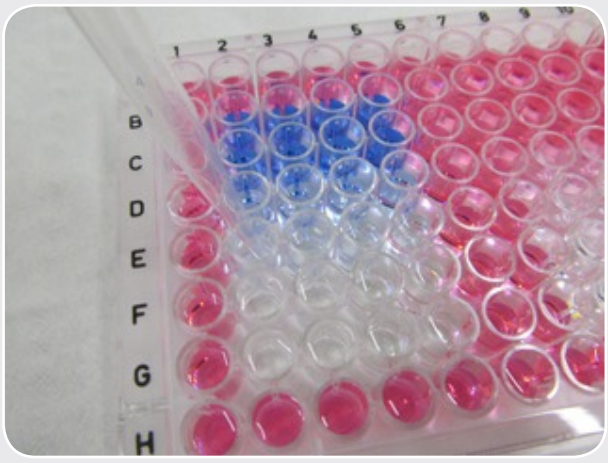
The complexity of real world events and the lack of knowledge about the future

As nanoparticles per se cannot easily be detected, it also becomes a problem to describe their interaction with the living environment. It is, however, a prerequisite for decisions to get significant results from *in vitro* and *in vivo* experiments about the short- and long-term toxicity of nanoparticles in humans, about their bio-distribution and clearance.

It is a fact that we still do not know enough about the nanoparticle-biology interaction. Nanoparticles, entering the body, associate with surrounding proteins which may differ when entering the body otherwise (lung, skin, blood etc.). Proteins will change the surface of the nanoparticles (protein corona) and will have influence on their recognitions by cells. Such interactions have to be considered in the interpretation of cytotoxicity data.

The uptake and defence mechanism of cells, the penetration of cell membrane and other barriers (air-blood tissue barrier (lung, skin), the lumen-blood tissue barrier (e.g., GI-tract) and the brain-blood barrier will be influenced by the way the particles will take when and after entering the body. *In vitro* tests may therefore give only a very

first indication of possible toxicity as in the actual methods interactions at systemic level are mainly neglected.
We should also get to know more about the clearing behaviour of nanoparticles to look into the future for long-term toxicity. Clearing of nanoparticles in the body depends on the type of particles and their location in the cell, the extracellular matrix, specific organs etc. Bio-distribution and clearing is described through the uptake by organs; however, one should also define the location from which particles might be cleared: a cell, the tissue, organs or even from the organism. Methods to track particles are very sophisticated, time-consuming and expensive. They can often only give a very limited and static picture of the event but they can rarely describe the dynamic of the particles' behaviour.



Determination of iron content in cells by prussian blue test.

What is known to be true and what is believed to be true?
Today we have two types of approaches – the bottom-up (science-driven) and the top-down (regulation-driven) – but both do not really fit for decisive strategies whether or not a nanoparticle should be further developed and may receive a kind of "safety label". The bottom-up approach seems to work fine from the scientific point of view, but causes problems for the regulation side and vice versa. Nevertheless, for both cases it would be important to receive the research outcome in a comprehensive and well-documented publication, so that results can be compared. However, even the scientific outcome is not published in a way that meta-studies can be carried out which may help to establish more general relationships between particle properties and cell behaviour. The published results often do not contain enough information about the experiments to compare results in a continuous way. This information gap caused by the absence of guidelines for proper presentation of materials and methods in most of the publications makes funding of nanoparticle research (including toxicology) inefficient and may even confound the appropriate measures in academia and industry as well. Properties of nanoparticles, responsible for possible toxic effects like size, shape, coating, charge density and possible biologic entities to be coupled etc., should be communicated in a publication, be it a supplement (if existing) or on a special web page, so that comparison between publications becomes possible. This would help researchers and companies to re-engineer the particle for higher safety (safety by design).
Especially in the medical field, in which cost and time of development and clinical tests is much more pronounced than in industrial products, the lack of efficient guidelines and the basis for elaborating industrial standards may be the limiting factor to go further in a development as the risks of failure in long-term clinical testing are too expensive. A more "standardized" nano-safety research would therefore be useful as problematic particle candidates could be recognized early enough and one could scope on more promising entities.
Risk assessment of nanomaterials, the improvement of methods for characterizing the materials and their association with the biologic environment are, of course, critical issues, one must, however, also emphasize that the *opportunities* these novel materials offer have to be balanced carefully with the *risks* they may cause. One may describe nanotechnology research as –"action research" in which "the researcher attempts to develop results or a solution that is of practical value to the people [...] and at the same time developing theoretical knowledge."[24]

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24 http://www.is.cityu.edu.hk/staff/isrobert/phd/ch3.pdf

Information about speakers and presentations

Professor Dr. Albert Duschl

Prof. Dr. Albert Duschl has been Full Professor of Biochemistry at the Department of Molecular Biology, University of Salzburg, Austria, since 2001. He received his PhD in 1986 at the University of Giessen and worked at the University of California in Irvine, the Max-Planck-Institute for Biochemistry at Martinsried and the Biocenter of the University of Würzburg. His specialty is the regulation of the human immune system, in particular conditions leading to misregulation, like allergy and inflammation in the absence of pathogens. Duschl is coordinator of the on-going FP7 ITN NanoTOES and work package leader for case studies in the ongoing FP7 NanoValid. In these and in other projects on nanosafety he and his group develop cell-based assay systems and explore molecular mechanisms responsible for toxic effects of nanoparticles.

Professor em. Dr. Peter Gehr

Prof. em. Dr. Peter Gehr was Chairman of the Institute of Anatomy at the University of Bern (retirement since August 1, 2010). In 1988 he became Professor of Anatomy at the Medical Faculty of the University of Bern. He was Visiting Lecturer at the University of Nairobi and Visiting Assistant Professor at the Harvard School of Public Health. He spent sabbaticals at the University of Western Australia, the National Jewish in Denver and the Harvard School of Public Health in Boston. Gehr carries an honorary degree of the International Independent University for Environmental and Political Sciences in Moscow (Dr. h.c.). For almost 30 years his research was on particle-lung interaction. During his last ten years until retirement he specialized in the interaction of nanoparticles with cells.

Professor Dr. Heinrich Hofmann

Prof. Dr. Heinrich Hofmann got his PhD in Material Science 1983 at the Max Planck Institute in Stuttgart and joined the R&D center of Alusuisse-Lonza Services AG (Neuhausen-am-Rheinfall) in 1985. He was first involved in the development of new alumina powders for ceramic application, then he developed a new titania stabilized zirconia powder and a pilot plant for a first fabrication of such powders in industrial quantities. In 1993 he joined the Swiss Federal Institute of Technology as Professor and Director of the Powder Technology Laboratory at the Department of Materials Science and Engineering. His research area includes the synthesis of nanostructured materials based on nanoparticles and the modification of surfaces with nanoparticles using colloidal methods for applications in medical and biological fields (drug delivery, hyperthermia, cell separation, biosensors), electronics and sensors, coatings of medical devices, turbine blades and paper. Hofmann is member of several professional organizations, member of the Swiss Federal working group “Nanoregulation” and member of various scientific advisory boards (Japan, China, Thailand).

Professor Dr. Yuri Volkov

Prof. Yuri Volkov received his MD from the 1st Moscow Medical University and subsequently a PhD in biomedical sciences at the Institute of Immunology, Moscow. He has been working at the Department of Clinical Medicine, Trinity College Dublin (TCD) since 1995. For a number of years his research interests have focused on leukocyte biology, mechanisms of inflammation and cell adhesion receptors functioning in immune defence and disease development. Over the recent years, as a Principal Investigator at the Trinity College's Institute of Molecular Medicine (IMM) and the Centre for Research on Adaptive Nanostructures and Nanodevices (CRANN), Volkov has formed a large-scale interdisciplinary alliance between the Schools of Medicine, Physics and Chemistry at TCD aimed at the development of new applications of nanomaterials for advanced research and medical diagnostics, nanoscale molecular imaging and drug delivery systems. He currently coordinates a large-scale EU FP-7 funded Consortium NAMDIATREAM (www.namdiatream.eu).

Dr. Peter Wick

Dr. Peter Wick heads the research lab for Materials Biology Interactions at the Federal Laboratories for Materials Science and Technologies (Empa) in St. Gallen. He studied and received his PhD in cell and molecular biology at the University in Fribourg (Switzerland). In 2002 he moved to Empa and began his research in nanotoxicology. His interest is to establish the methodology and gain the scientific knowledge necessary for a comprehensive understanding on how engineered nanomaterials influence human health. He is not only focusing on the uptake and dose-response relationships of nanomaterials but also on mechanistic *in vitro* as well as *ex vivo* studies in different human tissues. Wick is active in different projects funded either nationally or by the 7th Framework programme of the European Union. He is a member of the advisory board of the Swiss Action Plan on Nanomaterials and Precautionary matrix as well as Editorial Board Member of Nanotoxicology.

Dr. Margarethe Hofmann-Amtenbrink

Dr. Margarethe Hofmann-Amtenbrink (Ing.-grad. in Foundry University of Duisburg, Dipl.Ing., and PhD in Materials Science at Max Planck Institute Stuttgart and Technical University Berlin, Germany) is independent since 1987 with *Mat Search Consulting Hofmann* in Switzerland. She has managed various Swiss Societies and a Foundation, different research programmes of the Swiss Government and Foundations in advanced materials (Priority Programme Materials), in biomaterials (Gebert Ruef Foundation) and in biotechnology (AO Foundation Davos). For the past ten years Hofmann-Amtenbrink has been involved in research projects on nanoparticles for biomedical applications and initiated two EU Research Projects. She is member of several Advisory Boards in Germany and Switzerland (Helmholtz-Zentrum Geesthacht, Europäische Akademie Bad Neuenahr-Ahrweiler GmbH, Competence Center for Applied Biotechnology and Molecular Medicine, University of Zurich), Vice President of the Federation of European Materials Societies, FEMS and member of the Swiss Academy of Technical Sciences.

Acknowledgement

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Margarethe Hofmann-Amtenbrink and Heinrich Hofmann

Workshop participants

WS 1: Pierre Hugounen, Magdalena Priebe, Michael Riedicker, Corinne Jud; Peter Wick, Morteza Mahmoudi, Jürgen Höck, Cornelle Noorlander, Frank Schulze, Lionel Maurizi and Heinrich Hofmann

WS 2: Frank Schulze, Tania Cavaliero, Olivier Jordan; Pierre Hugounenq; Jan Mehlich, Magdalena Priebe; Marie-Gabrielle Beuzelin and Albert Duschl

WS 3: Yuri Volkov, Margarethe Hofmann, Peter Gehr and Azza Gramoun

WS 4: Jürgen Höck, Adrienne Sips, Tania Cavaliero, Jan Mehlich, Francois Roubert, Alessandra Hool and Peter Wick

Further information

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