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Nanoparticles in medicine: Current challenges facing inorganic nanoparticle toxicity assessments and standardizations

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Abstract

Although nanoparticles research is ongoing since more than 30 years, the development of methods and standard protocols required for their safety and efficacy testing for human use is still in development. The review covers questions on toxicity, safety, risk and legal issues over the lifecycle of inorganic nanoparticles for medical applications. The following topics were covered: (i) In vitro tests may give only a very first indication of possible toxicity as in the actual methods interactions at systemic level are mainly neglected; (ii) the science-driven and the regulation-driven approaches do not really fit for decisive strategies whether or not a nanoparticle should be further developed and may receive a kind of "safety label". (iii) Cost and time of development are the limiting factors for the drug pipeline. Knowing which property of a nanoparticle makes it toxic it may be feasible to re-engineer the particle for higher safety (safety by design).

Key words: Nanoparticles; Nanomedicine; Regulation; Toxicity; Standardization

Key issues in nanomedicine

Although scientists and clinicians have been engaged in nanomaterials and, more specifically, inorganic nanoparticles research for more than 30 years, the development of methods and standard protocols required for their safety and efficacy testing for possible human use is still work in progress. Inorganic nanoparticle use, especially magnetic iron oxide materials for imaging, over this period has been a particular focus, and their impact on human cell and tissue functions a compelling safety and toxicity concern. Assessments of the influences of particle size, morphology, surface charge and resulting interfacial protein adsorption on their interactions with tissues, uptake by lymphatic or blood components, and correlations with toxicity or safety risks certainly provide no consensus to date. In vitro methods and preclinical models to produce such correlations to human use currently lack validation and standards. Hence, without accepted approaches for assessing safety, translation of nanomaterials and nanoparticles may prove challenging as marketable biomedical products.

Under the auspices of the European Research Project NanoDiaRA (Development of Novel Nanotechnology Based Diagnostic Systems for Rheumatoid Arthritis and Osteoarthritis), funded by European Commission Framework 7, two workshops were organized on "Nanoparticles in Medicine: Toxicity Methods and Standards" in May, 2012 and September, 2013. Experts representing the following expertise were assembled: (i) nanoparticle synthesis processes and characterization from pure compositional and physical testing to investigations with the human components in vitro, (ii) regulatory issues surrounding nanotechnology and nanomedicine, and (iii) commercialization aspects required to take certified nanomaterials from laboratory-scale to GMP-certified biomedical product. Workshop discussions focused on the current plethora of diverse and invalidated research methods commonly

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employed in both academic and industrial nanoparticle in vitro and in vivo characterization. Lack of assay standards, comparisons and consistency frequently produce confounding results. Several critical issues involve the ability to translate inorganic nanoparticle from the many academic reports and studies to industrial scaling processes that comply with commercial quality systems, governmental standards, and regulatory contexts for human use. A more detailed report of the workshops is given on the Web page "Meeting summaries" of the journal, *Nanomedicine: Nanotechnology, Biology, and Medicine*.[1]

The many variations in reported investigations, often with vague descriptions of materials and preparation, storage, and analytical certification methods, prevent robust scientific comparisons of the diverse published results on seemingly related inorganic particle chemistries. Additionally, industrial standards are lacking for these systems: relevant legal guidelines and important definitions remain vague. Therefore, it is important to address the compelling need for improved, standardized assessments of inorganic nanoparticles and their toxicity in various biomedical uses, for coherence between scientific developments and corresponding health and safety regulations, and for defining prerequisites necessary for implementing and enforcing such regulations. These concerns lead to the following key issues:

(i) "Nanoparticle Properties and Characterization Methods": Inorganic nanoparticle size and shape, their physicochemical properties and, most importantly, surface and interfacial properties in biological milieu[2] that result in formation of the ubiquitous adsorbed protein corona on particle surfaces are proposed as critical parameters to measure. Importantly, these properties should be verified and followed to correlate and control their interactions with living systems throughout the entire product life cycle. This is the basis for the second key issue.

(ii) "Toxicity Assessment": Despite global proliferation of engineered nanoparticle research and production, reliable, validated high-throughput standardized methods are still needed for rapid assessment of their toxicity under various environmental conditionings, human routes of exposure, dosing to cell cultures and in vivo biological conditions. Correlations of in vitro cell and protein exposure results to in vivo host responses that are often uncertain and non-predictable to use for risk–benefit analysis. Furthermore, pre-clinical in vivo experiments and models necessary to best mimic a given dose–exposure situation for these nanoparticles in formulations appropriate for human uses have no current consensus, validation or standardization to date. These key issues represented in (i) and (ii) are again prerequisites of (iii) — the regulatory aspects for translating nanoparticle formulations to clinical use.

(iii) "Regulation": Government policies governing nanomaterials production and occupational exposures, environmental release, commercial product stewardship, and human exposure remain a critical part of the entire product life cycle for nano-enabled products.[3] Policy formulation and implementation must enable clear guidelines that govern interactions between nanomaterials researchers, developers, and regulatory bodies to together facilitate the responsible transfer of research results assessing toxicity (if any) to ensure product safety for industrial and medical users. This should be a living, dynamic engagement: research and development in nanotechnologies/nanoparticles for biomedical products are continuously evolving. New details about nanoparticle properties and toxicity with their associated implications for benefits and risks are continuously reported in scientific reports as well as consumer digests in the public media. Associated, evolving legal aspects surrounding these issues must also be considered and appropriate measures taken to provide both stability via responsibility to industrial developers for their future markets and also safety to the consumer in both proper use and exposure.

Considering the various discussions at workshops, conferences and recent publications, a general picture of the current situation and future needs can be constructed[4]:

(i) improved methodology and test tools for characterizing nanomaterials from research toward marketable versions and the throughout the product life cycles are necessary, covering the diverse manifestations and impacts of these materials on both human health and on the environment;

(ii) the assessment of possible risk should be harmonized between the main stakeholders in Europe, USA and if possible, other countries, regarding the spectra of current materials R&D and marketing for nanomaterials-based products;

(iii) nanomaterial-based products for industry and medicine should seek a common approach to safety and toxicology testing distinct in certain aspects from traditional new, soluble drug testing. This is especially important for those nanomedicinal products based on inorganic nanoparticles and for which conventional toxicology knowledge is often insufficient in routine pharmaceutical toxicology testing. Nanoparticle assays and their outcomes are not comparable with soluble molecule-based product assessments and must be treated differently;

(iv) improvements in regulating nanomaterials, especially nanoparticles, are necessary to address current ambiguities for industries that avoid the use of "nano-branding" in their nanomaterials-containing products if it is not specified as a marketing instrument;

(v) several current nanomedicinal products are based on re-invention or adaptation of formulating strategies for existing poorly soluble or insoluble drugs showing improved performance when encapsulated within lipid vehicles (i.e., liposomes) or as protein complexes, or in nanocapsules and organic (polymer) nanoparticles. Because of their complex synthetic preparation and composition, inorganic particles processed with various analogous organic or inorganic coatings and other possible conjugated biological moieties encounter greater difficulties in their translation toward clinical applications, depending on application and specific use.

Questions of scalable, reproducible synthesis, verification of reproducibility and homogeneity, equivalence, purity, analytical chemistry, biomedical performance, and safety validation in vivo, all critical for commercial financial and marketing assessments as well as a clear regulatory path forward, remain unanswered for most of these systems. No nanomaterials product will reach the market place for broad, general use and impact without an attractive commercial translation pathway that compels an industrial producer. Therefore, these mission-critical issues for nanomaterials biomedical translation must be addressed to reveal a viable path to product commercialization.[5]

Recommendations and still further questions

The development of new tools capable of better detecting requisite changes in cells placed in contact with the nanoparticles in biological milieu more sensitively is recommended. This includes methods for determining how many particles are in cell-contact and interrogation of several cell signaling pathways simultaneously. Without such sensitive tools, identifying the various interactions of nanoparticles in human tissues and their relevance to cell responses and cell uptake pathways over long-term (i.e., to ten years) exposures, safety and toxicity assessments and mechanistic profiling will remain extremely difficult and tedious, precluding reliable data interpretation and consensus building (i.e., as witnessed currently). The Swiss project VIGO[6] represents a good example for developing initial screening tests (four different endpoints each with at least two methods providing a risk ranking). The basis for the development of such tests and their demonstrated reproducible outcomes 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 reliable and efficient, clear guidelines must be written, agreed upon and enforced by journal editorial boards regarding the minimum amounts, types and quality of technical information delivered within publications for nanomaterials characterizations and assessments in vitro and in vivo. The authors are aware of the challenges to realize these demanding requests, but discussion about it will sensitize the research community about it, especially if they like a high citation rate. A first example along these lines is the DANA database.[7]

Additional open questions regarding nanomaterials toxicity tests include:

- Would nanoparticles exhibit similar consistent properties and interactions in different organs and cells? Does the history of the conditioning pathway of the particle in the body have any influence on these interactions?
- Are in vivo and in vitro studies with nanomaterials and cells confounded by cell sourcing? Secondary cell lines are often mis-identified or contaminated, and over-passaged without phenotypic validation.[8] Primary cells have diverse sources clouded by their different species-specific traits and unique "pathological and immunological histories", with equally vague phenotypes and validations reported. Hence, cell culture results can be very different even with the same nanomaterials. This source and study-based variation represents a general problem concerning all biological studies, not simply nano-relevant.
- Do nanoparticles have the same effect in an adult body as in a developing and growing younger body? How does age influence interactions?
- What control in vitro experiments can be used to detect and determine amounts and kinetics of particles reaching a cell surface? These are still not standard in toxicity tests. Dosimetry in vitro is controversial.
- What is the influence of particle porosity on particle transport and on protein adsorption (e.g., silica particles or coatings with silica have a porosity of >50%)?
- How can culture media's oxygen content and nanoparticle size distribution during cell testing be determined in real time?
- Can in vivo particle injection rate influence both the particle aggregation rate (locally high concentration, perhaps deposition), and also known uptake and metabolism in key filtration organs such as the liver, spleen, lung capillaries and kidney which may induce toxic by-products? Can steric stabilization alter toxicity profiles in vivo?
- Are all experimental parameters reported and used to assess particle toxicity really necessary to properly and reliably profile nanomaterials risk–benefit? What is their utility to predict risk versus benefit? How do toxicologists, biomedical researchers and biologists best use these data? Which are best for standardization? When do we have sufficient knowledge to reliably correlate particle properties with toxicity data and establish standards? Who stewards this process?
- Development of Standard Operating Procedures (SOPs): Who is responsible and who pays for their development, validation and assessment? Such tools are as important as standards.

Limitations

Clearly defined particles are readily accessible for model experiments. However, in the real world and under normal production procedures, particles show size and property distributions initially, and that change during biological experiments, and are therefore not easily "defined" any more (see Footnote 2). Lack of systematic know-how makes it currently impossible to predict nanomaterials behavior under different assay conditions. In addition, critical transitional size ranges that distinguish nano-type behavior from bulk materials behavior are unique to each material and its formulation, and generally poorly defined. The EU recommendation of 18 October 2011 on the definition of nanoparticles — "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 address this problem: more practical determinations are required for rational development to proceed using the "safe-by-design" approach. It appears that size limits for most of the physicochemical properties like increased band gap or magnetic properties are observed at particle sizes <10 nm whereas for biological properties this limit seems to be larger than >200 nm.[9]

The existing body of toxicity testing protocols and pool of uncorrelated results for nanoparticles represent an unsolved problem. Most reported results are based on nanoparticles insufficiently characterized or documented and therefore not

comparable between studies. Typical assays and tests are very often adapted from the known arsenal of toxicity tests used for soluble molecules. It is very interesting to note that on-going discussions[10] in the United States regarding new approaches for toxicity tests for chemicals and drugs as well as efforts to reduce the amount of animal testing for cosmetics do not currently extend to nano-toxicology assays. In Europe, these questions are less relevant because animal tests for cosmetic products are forbidden. Application of approaches similar to systems biology or computational systems biology is missing (as an example, see more sophisticated data analysis like fuzzy logic or Quantitative Structure Activity Relationships (QSAR) approaches used for analysis of complex systems). According to Bhattacharya,[11] modern toxicity testing should use approaches similar to systems biology. Further details and discussions to improve toxicity evaluation reside in Andersen et al.[12] It is interesting to note that in the EU FP7 research program, the last NMP call, published July 2012, now addresses these topics.

Some recommendations regarding ongoing research and regulation

Some important points to consider regarding particles, dosing, timing and methods include:

(a) 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 and allow comparison with other's published results;
(b) including the dosing algorithms in combination with nanoparticle size information, method of application and media would all better help to define the effects of such doses. In addition, doses to cells or tissues should not exceed levels by which cells only die because any effects of particle characteristics/functions are hidden by mass overload. Similar considerations are given to the time effect or study duration: What role does time play, what defines a "chronic effect" and how often are doses repeated to recapitulate chronic dosing? Comparisons of in vivo and in vitro studies are still plagued by dose and time variations, and arbitrary justification for each. Barriers for nanoparticle diffusion and penetration may also play a role in toxicity variations, and they should be defined and tested by using different particle types (size, material, coating etc.). Recommendations will also 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 analyzes the particles, the carrier and their combination.[13]

Regarding evolving policy and regulation, we like to point out that:

(c) Regulators and industry are in a "waiting situation" concerning the toxicological evaluation of nanoparticle medicinal products, so the initiative to establish best practices, standards and expectations has to be taken up by others (e.g., the government or possibly the research community).
(d) There are no explicit checklists yet. Regulatory bodies will not define such a checklist, as this is a political "minefield". Therefore, the users (producers and practitioners) have to develop such a list.
(e) The current concept of medical regulations is acceptable also for nanoparticles.
(f) Studies should be quality-controlled by defined criteria for technical detail, reliability, and relevance. They should focus on more predictive than descriptive data.
(g) Selection of the right models for addressing the right questions should be scientifically justified. In vivo and in vitro models used for nanoparticle-cell interactions should be validated.
(h) The definition of "nano" in medical applications is unclear and should be discussed further (considering the fact that nanoparticles > 100 nm — perhaps up to a few 100 nm — can easily enter cells).
(i) Dossiers and assays should be shared at least partially to distribute the general workload. It is foreseeable that with such an approach, IP problems will become an issue.

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

As nanoparticles per se cannot easily be detected in biological milieu or tissue, this complicates descriptions of their interactions with living environments. It is, however, a prerequisite for proper decision-making to obtain significant and consistent results from in vitro and in vivo experiments regarding short- and long-term toxicities of nanoparticles in humans, and about their bio-distributions and clearances.

Knowledge about nanoparticle-biology interactions, or even which interactions are most important over space and time, is currently insufficient and confusing. Nanoparticles entering the body associate with surrounding proteins that might differ between various sites of administration (e.g., lung, skin, vs. blood etc.). Protein adsorption is nearly instantaneous, serving to alter nanoparticle surface properties and dynamically changing its physical state (protein corona). This influences their activities with blood components (e.g., complement, platelets, leukocytes), recognition by cells, their cell uptake and oxidative stress-inducing potency. These interactions are key to the interpretation of cytotoxicity data, certainly in terms of mechanistic evaluations that might guide future improved nanomaterials designs.

The cell uptake and intrinsic reaction mechanism to particles, particle penetration of cell membranes and other physiological barriers (e.g., air–blood tissue barrier — nasal, oral, lung, skin), the lumen–blood tissue barrier (e.g., GI tract) and the brain–blood barrier will to varying degrees be influenced by particles properties upon entering the body. In vitro tests may therefore provide only an initial, incomplete, and perhaps distracting indication of possible toxicity potential, compared to in vivo exposures because the more complete interactions determining biodistributions at systemic levels are absent.

Improved understanding regarding clearance behaviors of nanoparticles is also essential to more accurately predicting their long-term toxicity. Biodistribution, pharmacokinetics and

pharmacodynamic properties for nanoparticles are in vivo quantities rarely predicted using in vitro methods. Particle tissue uptake then processing for clearance and elimination in the body likely depends on particle properties and their ultimate location in the cells of which tissues, the extracellular matrix, the specific organs etc. Host health history, pathology, co-morbidities and even genetic profiles are also likely to play roles in these particle physiological fates, yet are relatively unknown. Biodistribution and clearance are described through particle uptake by organs; however, particle organ locations (e.g., within a cell type, or extracellularly extravasated, which tissue, organs and clearance kinetics from the entire organism) might also be better predictors of particle toxicity. Current methods to track particles are very sophisticated, time-consuming and expensive. They can often only provide a very limited and static picture of locations and they can only rarely describe the dynamics of the particles' behaviors in entering and leaving biological components and host systems that react with toxicity markers.

What is known to be true and what is believed to be true?

Today we observe two types of approaches to nanomaterials development and application in health care — the bottom-up (science-driven) and the top-down (regulation-driven) — but neither of these is ideally suited to guide strategies for addressing whether or not a nanoparticle should be further developed for medical use and may likely receive a kind of "safety label" to move it forward. The bottom-up approach seems to work fine for scientific advances, but it also causes problems for the regulatory components, and vice versa. Nevertheless, both approaches would benefit from research results provided in comprehensive and well-documented forms so that results can be validated in parallel studies and compared. However, current scientific outcomes are often not published in ways that readily allow meta-studies to help establish more general relationships between particle properties and observed in vitro cell behaviors. Published results often do not contain information sufficient to allow experimental conduct to compare results in a consistent way. This information gap caused by the absence of guidelines and peer review expectations for proper descriptions of nano-materials and methods in most publications makes continued funding of nanoparticle research (including toxicology) inefficient and may even confound the appropriate future directions of such research in both academia and industry. Properties of nanoparticles responsible for possible toxic effects including size, shape, coating, charge density and possible conjugated or adsorbed biological components should be clearly analyzed and technically communicated in publications, be it a supplement or on designated Web page repository, to enable comparison. A repository for negative results is also valuable to avoid repetition. This level of detail should help guide researchers and companies to re-engineer nanoparticles for greater safety (safety by design).

Especially for biomedical industry where cost and development time for required clinical testing is much more pronounced than in non-medical industrial products, the present lack of direct, specific guidelines as the rational basis for establishing industrial standards may be limiting to stimulating innovation: as the risks of failure in long-term clinical testing are too expensive.[14] A "standardized" nano-safety research strategy with validated protocols and quality systems would therefore be useful for development and more reliable commercial vetting as problematic particle candidates could be recognized early enough to be eliminated, with resources focused on more promising candidates.

While nanomaterials risk assessment, methodological improvement for both materials characterization and their associations with biological environments are, of course, critical technical issues, new opportunities that these novel materials might offer must be balanced carefully against their risks to produce harm. Risk–benefit analysis is codified in most regulatory policies and protocols. An unmet challenge remains the assessment and prediction of risk — an imperative of high priority, but unknown opportunities in benefits for nanotechnology remain to be discovered as well. In this regard, nanotechnology research has already been described 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."[15]

Development

Costs and time associated with development are recognized limiting factors for the drug translational pipeline. Preliminary safety research has substantial intrinsic value to pipeline development by identifying problematic drug candidates early on, allowing rational elimination of these candidates to more rapidly focus on more promising entities. This same process might be also be applied to nanomaterials development for biomedical applications should a valid safety assessment process be established. Nanomaterials safety research could identify nanoparticle properties responsible for observed toxic effects, allowing rational re-engineering of particle properties for an improved safety profile (achieving safety by design). Ideally it would be most efficient to develop assays capable of pin-pointing a select, most problematic parameter without losing the desired material functionalities. In this case, a molecular understanding of toxicity mechanisms would be useful to provide specific technical guidance. This method would better enforce and inform materials development strategies for selective modification for example at a molecular or morphological level, supplanting current global toxicity screens incapable of discriminating mechanism and therefore forcing abandonment of entire materials in the name of safety.

Personalized profiling of nanomaterials effects

Safety research and profiling can also be used to identify persons, genotypes or phenotypes at risk of adverse reactions, including coagulation, allergic or autoimmune reactions. Identifying then excluding such patients from clinical trials and later post-approval from nanomaterials exposure or treatment would be highly beneficial and cost-effective.

Other future options could also exist, such as the development of personalized nanoparticle-based drug delivery, imaging and theranostic compositions that match patient response or sensitivity profiles or genotypes, less expensive production of drugs, early stage identification of patient adverse reactions and disease diagnosis due to enhanced patient sensitivity to nanomaterials.

These examples and perhaps other additional future developments already illustrate the value that nanoparticles provide to improving drug development efficiency. Focused research that provides new insights into associated nanomaterials risks and their mechanisms of toxicity relevant to human exposures should benefit nanomedicine.

Conclusions and recommendation

Considering the results of discussions about nanoparticles for medical applications and taking into account the opinion of the different stakeholders in the field of nanomedicine including the community working in the field of nanotoxicology, we come to the following conclusions and recommendation:

(1) *Establishing of laboratories which allows a GMP like synthesis including functionalization of nanoparticles foreseen for medical application*: Researchers which have developed promising systems should have the possibility to show the up-scalability of their processes and should be able to provide GMP produced particles for further investigation at clinical level. This would include also improved and internationally harmonized methodology and test tools for characterizing and assessing risks of nanomaterials from research toward marketable versions.

This conclusion and recommendation fits very well with the propositions elaborated by the European Technology Platform Nanomedicine in 2013 to build up new research infrastructure in Europe supporting the translation of nanomaterials including nano-characterization laboratory, pilot line for GMP manufacturing and a network of preclinical centers of excellence [16]

(2) *Regulations for the application of nanomaterials*: especially in the field of the various organic and inorganic nanoparticles, regulation should be established and implemented as soon as possible to encourage further translation of nanomedicine toward acceptable industrial used products.

For example in the field of inorganic nanoparticles for medical applications, only few of these materials are under development and show an interesting potential for applications (iron oxide, silica, gold). Focusing on these materials could accelerate the regulation process substantially.

Following these two recommendations the development of nanomaterials for medical applications could be accelerated and economic risk for the pharmaceutical industry would be reduced substantially. This would encourage them to participate in this promising technology.

References

1. http://nanomedjournal.com/content/meetingsummaries.
2. Grainger DW, Castner DG. Nanobiomaterials and nanoanalysis: how to improve the nanoscience for biotechnology. *Adv Mater* 2008;**20**:867-77.
3. Gwinn MR, Sokull-Klüttgen B. Regulation and legislation. In: Fadeel B, Pietroiusti A, Shvedova A, editors. *Adverse Effects of Engineered Nanomaterials: Exposure, Toxicology, and Impact on Human Health*. 1st edit. London Academic Press; 2012. p. 97-120.
4. Stern ST, McNeil SE. Nanotechnology safety concerns revisited. *Toxicol Sci* 2008;**10**:4-21.
5. Duda GN, Grainger DW, Frisk ML, Bruckner-Tuderman L, Carr A, Dirnagl U, et al. Changing the mindset in life sciences toward translation: A consensus. *Sci Transl Med* 2014;**6**:264cm12.
6. http://www.ccmx.ch/projects-amp-partners/past-projects/ [April 2015].
7. http://www.nanoobjects.info/ [April 2015].
8. Perkel JM. Curing cell lines. *BioTechniques* 2011;**51**:85-90.
9. He C, Hu Y, Yin L, Tang C, Yi C. Effects of particle size and surface charge on cellular uptake and biodistribution of polymeric nanoparticles. *Biomaterials* 2010;**13**:3657-66.
10. Oberdörster G, Stone V, Donaldson K. Toxicology of nanoparticles: A historical perspective. *Nanotoxicology* 2007;**1**:2-25.
11. Bhattacharya S, Zhang Q, Carmichael PL, Boekelheide K, Andersen ME. Toxicity testing in the 21st century: Defining new risk assessment approaches based on perturbation of intracellular toxicity pathways. *PLoS One* 2011;**6**:e20887.
12. Andersen ME, Al-Zoughool M, Croteau M, Westphal M, Krewski D. The future of toxicity testing. *J Toxicol Environ Health B Crit Rev* 2010;**13**:163-96.
13. Nanomaterials: Commission proposes case by case approach to assessment. http://europa.eu/rapid/press-release_MEMO-12-732_en.htm [April 2015].
14. Grainger DW, van der Mei HC, Jutte PC, van den Dungen JJAM, Schultz MJ, van der Laan BFAM, et al. Critical factors in the translation of improved antimicrobial strategies for medical implants and devices. *Biomaterials* 2013;**34**:9237-43.
15. http://www.is.cityu.edu.hk/staff/isrobert/phd/ch3.pdf [April 2015].
16. NANOMEDICINE 2020. Contribution of Nanomedicine to Horizon 2020. www.etp-nanomedicine.eu/ [April 2015].
17. Full list of participants is given in http://nanomedjournal.com/content/meetingsummaries.