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Nanotechnology in medicine: European research and its implications

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Summary

In this study, we explore and discuss nanoparticles and nanoscale materials and their use in medicine (nanomedicine) and pharmaceutics (nanopharmaceutics). The study is aimed at shedding light on this highly multidisciplinary research field and at examining the influence of research funding, industrial applications, and legal and regulatory frameworks on the research in this field, a clear understanding of which is essential to efficiently support the translation of research findings into industrial and clinical applications and to enable access to a larger society.

Key words: *nanotechnology; nanomedicine; nanoparticle; translational research*

Introduction

For over thirty years, intensive research has been conducted in the field of nanotechnology worldwide, in particular, in the area of nanomaterials. In the last decade, products containing nanomaterials, such as textiles, cosmetics, and recently developed products for biomedical applications, have entered the market at an increased pace. However, specific "nano-effects" are not always emphasised on for such products. In the late 1970s, C.G. Granqvist and R.A. Buhrman [1] discovered nanoparticles and identified their specific properties, calling them "ultrafine particles". These particles were mainly discussed because of their occurrence as natural particles, or they were considered by-products of industrialisation with regard to their contribution to air pollution and their impact on health. The first publications dealing with "engineered nanoparticles" were produced by the Australian and Swiss pharmacists J.J. Marty, R.C. Oppenheim, and P. Speiser in 1978 [2, 3] and by U. Schroder from Lund University in 1986 [4], who suggested the use of inorganic nanoparticles for drug delivery. Only inorganic or organic particles of nano-sizes, but not molecules or biological entities (like viruses), are considered nanoparticles even though these constructs are also in the size range of nanometers. This is due to the scientific interest in the size-related physical and chemical properties that differ significantly from those observed in particles with larger sizes or in bulk materials.

Over time, research and development of nanoparticles has gathered wide attention and interest, and various research initiatives have been launched worldwide (e.g., the National Nanotechnology Initiative in the USA in the 1990s, the European COST Action 523, the European Frameworks, and national activities at a global scale).

Because of the vast response that nanoparticle-related research and products have received in society, the adjunctive ethical, legal, and social aspects – especially the opportunities and risks of nanoparticles and nanotechnology – are investigated and discussed in this paper. Diagnostic and medical applications are particularly important areas to consider as nanotechnology offers many promises of improved treatment and enhancement of the quality of life of patients with respect to several diseases.

However, there are several concerns worldwide about nanotechnology and its possible toxic side-effects, and the legal framework applicable to the use of these technologies needs to be continuously explored and re-evaluated. Inconsistencies in definitions and standards in such an emerging and increasingly prominent field like nanotechnology could lead to uncertainties in patenting and regulatory bodies when applying research findings to produce tangible products.

Starting with a discussion regarding the definition of nanotechnology, this paper is focused on the describing the use of nanotechnology in medical applications, discussing the relevance of the novel properties of nanoparticles for medical uses, and, finally, we aim to highlight the important aspects that influence the current research and development as well as to disseminate the findings of the research in this interesting field.

What is nanotechnology?

In 2003, a group of eleven experts in the field of nanotechnology, philosophy, and risk assessment under the auspice of the Europäische Akademie Bad Neuenahr-Ahrweiler GmbH discussed six existing definitions of nanotechnology and stated that "*the lessons learned from the*

six definitions of Nanotechnology […] are that the existing definitions of Nanotechnology sketch a fuzzy picture of what Nanotechnology is about. These definitions are hardly usable in concrete decision situations, and […] the respective purposes of definitions are essential in debating about their appropriateness and adequateness." They provided their own definition of nanotechnology, thereby particularly emphasising the fact that properties of nano-sized substances should not have any equivalents in the macroscopic world. Having specific size-dependent physical properties such as magnetic, chemical, electronic, optical, or thermodynamic properties, and being defined by a finite number of constituents make nanoparticles unique and result in their inability to be described by classic physical laws but only by quantum mechanics. As Schmid et al. explain [5]: *"Nanotechnology is dealing with functional systems based on the use of sub-units with specific size-dependent properties of the individual sub-units or of a system of those."*

An example of a functional system could be a titanium oxide nanoparticle in the anatase form as, with the size of the crystallite, the wavelengths of absorbed light change. Smaller particles, less than 10 nm in size, show a larger band gap and absorb light of higher energy, like UV light. Such particles are used today in products like sun-creams because of their strong UV light-absorbing capabilities. On the other hand, the commercial use of titanium oxide particles of sizes between 10 and 100 nm, such as white pigments in paints, papers, or toothpastes, does not qualify them as functional systems, and, therefore, they cannot be considered nanoparticles according to the definition given by Schmidt et al. [5]. Such a strict definition can create a number of issues as it can segregate a major area of research activities and related products from the field of nanoparticles, nanotechnology, and other research areas dealing with nano-sized particles. This may also create problems for regulators as they have to distinguish between nanoparticles that fit the definition provided by Schmid et al. [5] and other particles that are only nano-sized, or ultra-small (fine) particles. Interestingly, the EU commission does not define "nanotechnology" but the term "nanoparticle" in a very precise manner: it states that a material is a nanomaterial if more than 50% of the particles in this material have at least one dimension between 1 nm and 100 nm [6]. Alternatively, it is also a nanomaterial if it has a specific surface per unit volume larger than 60 m^2/cm^3. According to this definition, graphene, naturally occurring and incidental nano-sized materials, as well as manufactured particles, are also included in nanomaterials; aggregates and agglomerates of such particles are also included.

Another major area of nanotechnology is based on effects arising from the high surface-to-volume ratios of nanoscale materials, such as the catalytic effect. For example, researchers at Mobil Oil Co. were able to synthesize nanostructured crystalline zeolite that, with a pore size of 0.45–0.6 nm, enabled the control of selectivity in petrochemical processes at the molecular level. Today, zeolite catalysts are used in the processing of petroleum and chemicals at an industrial level. Metal nanoparticles supported on oxides have become fundamental components of several devices. Their nanosized structures with new chemical and physical properties often result in a higher reactivity of these surfaces relative to their bulk counterparts [7]. The high surface-to-volume-ratio in nanoparticles can lead to changes in the crystal structure and thus reduce the melting temperature. Subsequently, the chemical and optical properties of the material are influenced.

Nano-effects in medical and pharmaceutical applications

Overview

Materials used in particulate form for medical applications in diagnostics and in drug delivery can be divided into the following sub-groups: (a) liposomes, (b) polymeric micelles, (c) polymer–drug conjugates, (d) dendrimers, (e) oil nanoemulsions, (f) mesoporous silica nanoparticles, and (g) iron oxide nanoparticles [8]. Javed [9] defines such constructs as *nanopharmaceutics, "because of their size effect, are capable of altering the properties of a drug, including their bioavailability, biodistribution and pharmacokinetics."* Alternatively, Bawa et al. [10] describe nanopharmaceuticsas colloidal particles of 10 to 1000 nanometers with properties fundamentally different from objects of macroscopic sizes. In their description, they distinguish between "*therapeutic molecules*" which represent a nanoformulation themselves, from those that are coupled to a nanoparticulate carrier. Their published work points specifically at Doxil®, Abraxane®, Diprivan®, DaunoXome®, Estrasorb™, Macugen®, and Amphotec® as nanopharmaceuticals, which were approved by the FDA between 1989 and 2005. These were approved *"according to pre-existing laws and without any special testing (e.g., with respect to pharmacokinetic profiles)"*. In addition, approvals of new nanodrugs and "nanoreformulations" are often granted for complex combination products (carrier plus therapeutic molecule) but the safety of the carrier, the therapeutic molecule, and the interaction between these has to be studied and approved carefully in view of potential health and environmental risks.

Essentially all types of inorganic and organic nanoparticles, including liposomes, can be used as carriers for therapeutic molecules and can be described as "complex combination products." However, even without molecules attached or included, they have an affinity towards various proteins that are always present in biological systems. Such binding of nanoparticles to proteins takes place when used for *in vitro* investigations (e.g., immune assays) and especially when applied *in vivo* via the lymphatic system, the blood system, or any other system in the body. This property of initial rapid adsorption of proteins on biomaterial surfaces has been known since the early 1970s when the blood compatibility of biomaterials was first investigated. In the case of nanoparticles, the surface is much larger than that of macro-devices. In addition, the particles are free-floating and, depending on their passage to the targeted cell, tissue, or organ, they may change their adhesion behaviour. The surface coating (composition, charge, confirmation), morphology of the particles, and size are crucial to the adsorption of proteins or other molecules. Assuming that such nanoparticles can bind to or enter cells, cell compartments,

or even the nucleus, they could potentially be used as therapeutic agent without further therapeutic molecules needing to be attached. Such a nanoparticle could then potentially act as a kind of "molecular therapy." (A frequent opinion is that molecular therapeutics target diseases at a molecular level and thus modulates the disease at its roots.) However, the techniques for the manipulation of the surface proteins are not yet sufficiently elucidated to allow the nanoparticles to be used as such at this stage.

The most thoroughly investigated nanomaterials today are gold or iron oxide nanoparticles. The latter are applied in medical diagnostics as contrast agents for imaging of the liver, liver metastases, lymph nodes, cardiovascular diseases, and inflammatory diseases such as arthritis. They may also be used in therapy as vectors for the transport of drugs and in the use of hyperthermia in the treatment of cancer. The latter makes use of the intrinsic heating effect of magnetic nanoparticles when exposed to an external magnetic field [11]. Most of these functional systems are currently still at the research stage or used in clinical trials.

Superparamagnetic iron oxide nanoparticles

Superparamagnetic iron oxide nanoparticles or SPION – a term also used in the following sections and which describes only nanoparticles of less than 30 nm and are present as γ-Fe_2O_3 (maghemite) and Fe_3O_4 (magnetite) – are particularly important to the health sector. It is important to note that magnetite has improved biocompatibility, since the iron is completely oxidised (Fe^{3+}), thereby strongly reducing the formation of reactive molecules that could damage the cells (oxidative stress). Nevertheless, SPION need an additional coating with polymers that allows for a longer residence time in the blood circulation and the attachment of biomolecules such as antibodies. This additional coating is an important feature essential for magnetic resonance imaging (MRI), as it prevents the particles from forming agglomerates due to the magnetic interactions, which in turn could lead to problems such as thrombosis or artifacts in imaging. Two superparamagnetic contrast agents are clinically approved, namely ferumoxides (Feridex® in the USA, Endorem™ in Europe) with an overall particle size (including polymers) of 150 nm, and ferucarbotran (Resovist®) with a size of about 60 nm. Both types of particles are coated with dextran and are approved specifically for MRI of the liver.

With regard to hyperthermia, heating of the particles is achieved by an alternating magnetic field through the Néel relaxation process. As the relaxation time of the nanoparticles has to be in the range of medically acceptable frequencies (100–300 kHz), the size of the particle must be adjusted as precisely as possible to values between 15 and 25 nm. Under such conditions, the applied magnetic field strengths should be below 6000 A/m (corresponding to about 10 x 10–^{3} Tesla) to prevent the formation of uncontrolled "hot-spots" of high temperature in the body.

Examples of the application of SPION nanoparticles and nanoformulations in medical therapy are being tested in clinical trials or are already on the market, including drugs used in cancer therapy. The nanoparticles are embedded in a rigid polymer shell, called beads, and injected directly into the tumour. The temperature that is generated by applying an alternating magnetic field is high enough to cause the death of the tumour. Again, the scientific research is still largely in its infancy, even though some companies are conducting tests in clinical phases I–III for such particles and related equipment (http://www.magforce.de/).

In addition to hyperthermia and medical therapies, a third essential application of SPION is the separation of proteins. All these applications often have a functional and biocompatible coating of the particles in common. Researchers have used different natural or synthetic polymers for the coating, but also inorganic materials such as silica or gold. The most prominent coatings of SPION are dextran, polyethylene glycol, and polyvinyl alcohol (PVA). In the case of PVA-coated particles, biocompatible PVA functionalized with amino or carboxyl groups are used, which not only serve for charge regulation but also as anchor points for further attachment of peptides and proteins, including antibodies and fluorescent dyes.

Gold nanoparticles are being used primarily for medical analysis, utilising the effect of the local plasmon resonance, which is based on the interaction between light as an electromagnetic wave and the free electrons (electronic conduction) in the metal. When light hits the gold nanoparticles, the free electrons oscillate together in the frequency of the light from one side of the nanoparticle to the other. The size of the particle determines the time required for the electrons to oscillate. This way a dipole is formed and light of a corresponding wavelength is absorbed. If these wavelengths are in the visible light range, the effect can be observed with the naked eye. Suspensions of small (10 nm) gold particles absorb blue light and the compound turns red, as can be observed for red church glass in which the same effect can be visible. This effect can now be used for rapid analysis of DNA defects [12]. Gold particles are coated with a single strand of DNA complementary to the corresponding DNA encoded in amino acid sequences that are typical of the investigated disease. If a patient has the disease, there is a highly specific binding of the patient's protein/peptide sequence to the complementary portion of DNA already bound to the particles. Thus, this property of nanoparticles can be used for simple, safe, and cost-effective disease detection. Other applications incorporate gold particles in spectroscopic analytical methods, as discussed above. Locally strong electromagnetic fields can be generated to stimulate molecules for spectroscopic studies, so that the detection limit can be improved significantly (example: Surface Enhanced Raman Spectroscopy).

Quantum dots

A third effect of these particles that could be of importance in medicine is the phenomenon of quantum dot semiconductors, which have a band gap between the valence band and the conduction band. In a crystal with a diameter of less than 10 nm, this band gap increases, and therefore the wavelengths of both the absorbed and the emitted light (blue shift of fluorescence) increase as well. Such particles may be used as contrasting agents for optical microscopy, where for each variable colour another antibody is anchored on the particles and thus various components of the cell can be observed simultaneously. For photothera-peutic applications where a very precise adjustment of the

wavelength of light is necessary, such particles can also be used. The advantage of inorganic semiconductor nanocrystals in the form of quantum dots is their high chemical and optical stability compared with organic fluorophores. Unfortunately, however, most quantum dots that emit light in the visible range are toxic and require an additional dense inorganic coating of the surface. In most cases, this is made of silica and an additional organic coating to improve the biocompatibility [13].

Nanomedicine and nanopharmaceutics – new fields in research and industry

Many definitions, many interpretations

Today, the term nanomedicine is essentially derived from the definition of nanotechnology. In 2005 the European Science Foundation (ESF) in its report titled *ESF Forward Look on Nanomedicine 2005* [6] issued the following statement: "*The field of 'Nanomedicine' is the science and technology of diagnosing, treating and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body.*"Problems may arise with regard to the materials, technologies, or products that can be labelled as "nano" and in deciding as to which of these materials (of sizes greater than 100 nm) have been wrongly classified as "nanoparticles". Etheridge et al. [14] conducted an evaluation of clinical trials in which 44 different nanoscale or nanostructured materials were defined in advance based on their relevance in nanomedicine. A search of related citations of clinical studies was performed. Around 800 clinical trials with potential nanomedicinal applications or products were discussed from the viewpoint of evaluating the types of products, their application in development, or, if already on the market, their current use. Trials were conducted for 141 unique applications and products; 38 already approved products were investigated for new applications and the remaining ones for new innovative products. Most of the applications were cancer-related, followed by infectious diseases. The diagnostic area, i.e., *in vitro* testing and *in vivo* imaging, comprises mostly nanoparticle application while the device area covering implant coatings and bone substitution often uses larger nanoparticles (>100 nm) and microparticles. Both investigational and commercial applications show clearly that in therapeutic applications the investigational products predominate even in the most cited area of cancer, while for diagnostics/devices a rather different picture can be seen. Etheridge demonstrated that around 60% of the reviewed investigational and commercial products have a size greater than 100 nm and therefore they would not be considered as nanomedicine products if the definitions of the EU or the guidelines of the FDA [15] were to be used.

This also provides an indication of the challenges, which are related to the legal barriers for nanoscaled products in medicine – risk evaluation, toxicity evaluation, and formulations. To incorporate nanomedicine-based investigational products into marketable applications, it does not seem necessary to encourage scientists to recreate more nanopharmaceutics and nanoscale or nanostructured materials; instead, it is essential to evaluate the existing materials, to increase the understanding of the underlying physicochemical and biological mechanisms, and to discuss the various options for translational research to be undertaken by centres for clinical trials and companies interested in the development of such materials. Such an approach is only possible if scientists and engineers, clinicians and the management of companies develop common research standards and make commitments in first-class research to the characterisation of materials, standards for toxicity tests depending on the various materials, and standards for publications, i.e., for materials, processes, and methods used for high-quality research and development. It is further recommended to stress the importance of discussing the outcomes of tests showing no toxicity effect [16] and that company-based research, which is partially funded by governmental or societal monies, should be published (proprietary reasons come first but after a certain time the information should be open to the public). Finally, as mentioned by Bleeker et al. [17], it is essential to introduce definitions that cover not only the requirements for industrial nanoparticles, but also for nanoproducts as defined by EU and European Medicines Agency EMA.

Nanoformulations as "re-inventions"

The advantages of nanotechnology, nanoparticles, or nanomedicine over conventional technologies emanate mainly from new features related to their considerable small sizes. However, such sizes may exceed 100 nm, especially in nanomedicine when dealing with larger molecules, which also have interesting characteristics.

The main drivers to translate nanotechnology into medicinal products such as nano-based drugs are the potential solutions offered by miniaturisation to fundamental problems like poor water solubility of drugs. In conventional processes drugs need toxic organic solvents [18] and show a lack of target specificity [10]. Existing pharmaceuticals, however, can be "re-invented" with new nanoformulations (re-use of drugs in nanoformulations when patents expire) and thus reduce the cost of drug discovery, design, and development. An example is the recently FDA-approved "*solvent-free formulation of paclitaxel for the treatment of metastatic breast cancer that utilises 130–nanometer albumin-bound (nab™) technology (Abraxane®; nab-paclitaxel) to circumvent the requirement for solvents*" [19].

An interesting approach to underscore the various requirements for a nanoparticulated drug (or "nanodrug") strategy is presented by Y. Liu [20]. The author distinguishes between active and passively targeted nanodrugs, whereby the passive targeting to tissues or organs is due to the enhanced permeability of barriers and thus offers nanoparticles and nanodrugs a more accessible route to reach the targeted area. Most of these products are taken up by the reticulo-endothelial system and the nanodrug is released at the targeted site due to changes in certain physical conditions like temperature or pH (thermal-sensitive and pH-sensitive polymers). Targeting ligands (antibodies, lectins, sugars, hormones, among others.), which identify the cell/tissue receptor, can enhance the active targeting and by using external excitation conditions (such as IR light and a magnetic field) can also release the drug, thus improving

efficacy. Nevertheless, the basis for such considerations is that the physical space of the drug is reduced, that its physicochemical and biological properties are changed due to its small sizes, and that it can enter cells or pass barriers more easily (e.g., the blood-brain barrier) and thus enhance the bio-availability of the drug.

Liu discusses three types of drug-loaded nanoparticles. The first refers to the "*common drug-loaded particles*" in a nanoformulation, which creates an unsteady physicochemical state; these are readily degraded. The second type is the "*controlled-release drug-loaded particles*," having a release process that follows a specific law, and these particles are designed to release a specific concentration of the drug for a specific tissue. Type 3 covers the "*targeting drug-loaded particles*," which are the most specific type of nanoparticles that may have different affinities to different cells and can be activated under the influence of external forces (e.g., magnetic forces). The companies involved with nanodrugs chiefly follow the first approach of "*common drug-loaded particles*" in a nanoformulation, as shown by R. Gaspar in a report entitled *Nanomedicines Challenges and Opportunities in a Global Development Environment* [21]. The author distinguishes between the different forms in which nanodrugs can be delivered: (i) nanocrystals (already marketed as Rapam®, Emend®, or Megace®, and other products that are currently in clinical trials, such as silver or paclitaxel); (ii) liposomal and lipid products, many of which are currently in clinical development, while others, such as doxorubicin for cancer or amphotericin B for fungal infections, are already marketed; (iii) polyethyleneglycol (PEG)-based polymer-protein conjugates (PEG-Interferon alpha 2b (marketed)) and polymer-drug conjugates which are in clinical phases (paclitaxel). "*Controlled-release drug-loaded particles*" or "*targeting drug-loaded particles*" like iron oxide nanoparticles for MR imaging are still in development or in clinical trials. Their processing is not comparable with the other nanodrugs and mainly based on conventional processes described earlier. The nanoparticles are partially biodegradable (for example, SPION in the liver are biodegradable but not in the kidneys) and they require the drug to be loaded on the surface. Therefore, their behavior in the human body (long-term toxicity, biodistribution, and degradation) is more questionable and still under investigation.

Nano in translation

What conclusions should be drawn by the research community in the field of nanotechnology and nanomaterials? This question is particularly essential for research funding in the field of nanoparticles. First, it is important to recognise the achievements of researchers and research funding organisations in having been able to take the complex field of nanotechnology from fundamental research toward development and innovation in only two decades. It is not the nanoparticles or nanotechnology research *per se*, but their use in marketable applications (innovations) which are now at the forefront. The shift of nanotechnology and nanoparticle research toward the development of applications means that funding is connected increasingly tight to industrial sectors. These include the energy sector (solar panels, batteries, carbon nanotubes, reinforced composites for wind turbines, or lightweight constructions), the health sector for contrast and therapeutic agents used for the great challenges such as central nervous system disorders (Alzheimer's, Parkinson's, and multiple sclerosis), various cancers, inflammatory and autoimmune diseases like rheumatoid arthritis, and cardiovascular diseases. Information technology also depends on micro- and nanotechnology like nanostructured polymer films known as organic light-emitting diodes, transistors based on nanoscale printing processes, and nanometer-scale magnetic tunnel junctions enabling very fast and effective magnetic random access memory (MRAM). In other words, nanotechnology has become the "enabling technology" for which it was developed in the first place. Nanotechnology today is accepted by a large sector of the industry and, when fully developed and applied, it has potential to provide products of greater value if production processes can be accelerated or become less expensive. Therefore, scientific innovation in this area is turning into an industrial innovation and the label "nanotechnology," which initially helped to increase research-funding opportunities, now enables researchers to meet the real needs of the industry and consumers. The large number of patents that has been filed during the last years can best demonstrate the translation of scientific research into marketable innovations.

Dissemination of nanotechnology outcomes

Today, the awareness of society to the potential dangers of the use of new technologies is higher than ever before, and questions that revolve around health and environmental influences need to be answered convincingly. Nanoparticles play an essential role in a variety of products across all industries, including the health sector through applications such as biomaterials, drug delivery, and nanodevices. The proportion of publications in the field of nanotechnology that deals with various aspects of nanoparticles, covering synthesis, modification, and application of these nanoparticles has increased about three times within a decade, whereas the number of scientific publications dealing with the issues of the toxicity of nanomaterials, particularly nanoparticles, has increased 30 times. In connection with possible health risks, nanoparticles and nanotubes and their applications are about one and a half times more often cited in *PubMed* than articles dealing with nanotechnology-based sensors or electronics.

In the area of nanoparticle toxicity in medical applications, the quality of the research naturally plays a crucial role in producing results that are meaningful for product development. It appears that nanoparticles are often treated like molecules and therefore existing methods, which are applied with great success in assessing the toxicity of chemicals and drug development, are often accepted without any further examination of their applicability in studies to the toxic effects of nanoparticles. Consequently, about 70–80% of the literature covering the toxicity of nanoparticles may be deemed unavailing, as explained by Harald Krug of the Swiss Federal Laboratories for Materials Testing and Research (EMPA), St. Gallen, Switzerland. He discussed this

at the Spring Conference of the European Academy in Berlin on 19, 20 April 2012, a fact which was recently confirmed by Patrick Boisseau, President of the European Platform Nanotechnology at the annual meeting 2013 in Grenoble, France. To obtain results that are not influenced by issues like the interactions between various nanoparticles and contaminants or solvents that may be toxic due to the system used or in themselves – for example, when using MTT assays[3], or the extremely high doses of nanoparticles used in animal experiments – it is necessary to carefully characterise the nanoparticles and to select test methods which are suitable and validated for such investigations. (An MTT assay is a colorimetric assay for measuring the activity of cellular enzymes. It was found that the colourof the observed particles interfere with the MTT test.)

Considering that nanomedicine deals with nanodrugs, which are small-sized versions of already approved drugs, and nanoparticle-based delivery systems based on inorganic nanoparticles that are still under investigation, it becomes clear that the quality of the disseminated results of research in this field can only be improved by high-quality studies and by reliable and reproducible research. An important and pioneering step towards increasing the quality of the research methods, tools, and the characterisation of nanomaterials has been undertaken in the EU infrastructure project "QualityNano." Its core aim is the creation of a 'neutral' scientific and technical space in which all stakeholder groups can engage, develop, and share best scientific practice in the field.

Journals and Editorial Boards should place more extensive emphasis on the importance of details in describing materials and methods and allow reporting – especially in toxicity investigations – of important outcomes, for instance, when stating that a nanomaterial is safe. Krug and Wick [16] presented an action plan encompassing guidelines for future publications that allow for the comparison of results between studies, and for discussions and arguments to be written in a style comprehensible by both experts and laypersons. If these recommendations were to be followed, it would also imply that part of the global financial funding for nanoparticle and nanomedicinal research is currently not efficiently used, as the outcome of some of the research is questionable and not always reproducible.

Conclusions

Research in nanotechnology and nanoparticles is globally funded and remains an influential "trendsetter" in research and development because of its multidisciplinary nature and because it interconnects multiple technologies. Nanomedicine is an innovative sector in which nanotechnology and nanoparticles are used to improve the quality of life of patients by providing earlier and more specific diagnostic methods and better-targeted, less invasive methods of treatment. The definitions of nanotechnology and nanoparticles, which imply specific size-related properties, have focused mainly on size (<100 nm), while nanopharmaceuticals use even larger sizes when dealing with so-called nanoformulations of already approved drugs (up to micron size). In this case, the definition of nanomedicine, which is based on the definition of nanotechnology and nanoparticles being less than 100 nm in size, cannot be applied.

After more than 20 years of intense nanotechnology research, there is a clear trend towards application-oriented research, especially in the biomedical field where versatile tools for each application are no longer available. It becomes necessary to define the targeted field very early in the research process and to find a marketable solution very rapidly. It is more useful and less time-consuming to find new nanoformulations for approved drugs than to start afresh with inorganic nanocarriers for targeted drug delivery. This is particularly the case for large pharmaceutical companies, which need a continuous revenue growth to create shareholder value in a sustainable way. For diagnostic devices and their nanoparticulate enhancers, the market is even more challenging as the yearly increase in the number of device manufacturers is exceedingly low.

Aside from these market-based views, there are still safety issues to be taken into account. Research has focused chiefly on the risks and the ethical aspects of using nanoparticles in medicine and other application sectors. The outcome of toxicity studies can be crucial and determine the direction of further research and development towards a possible product. If toxicity studies become an important source of concern, it is recommended to reassess some of the applied methods, to pay more attention to the material descriptions and characterisations and to their impact on the results. The various action plans in place and further discussed on national, European, and international levels seek to standardise the research methodology and by this to help improve the exchange of results. At national and European levels, academic and industrial development in the implementation of research findings from the laboratory to the clinic ("bench to bedside") is still hampered. This is because the development of methods, the translation of laboratory results to those of up-scaled materials and long-term toxicology studies, and the standardisation of regulatory testing require more infrastructure and guidelines that are globally available and harmonised.

The initiative of the European Technology Platform (ETP) Nanomedicine to establish a new "Nano-characterisation" infrastructure [22] is a first important step in this direction. The proposed centres or companies that could help scaling up nanomaterials under good manufacturing practice (GMP) control and regulatory standards, which otherwise can only be produced in very small quantities under laboratory conditions, would help to accelerate the technology transfer.

In conclusion, research with nanoparticles and nanoparticulate systems used in diagnostics and therapy is well funded worldwide and results are widely disseminated through a broad range of publications and patents. On the other hand, large pharmaceutical companies have had to deal with the issue of the so-called "patent cliff" for the past few years: patents are bound to expire shortly and there is a risk for sales to decrease dramatically. Nanoparticulate pharmaceutical systems based on well-known formulations may help, especially if not hindered by legal issues. However, in the case of combinatorial products like inorganic nanoparticle-based drugs or nanoparticle-based biomarker combinations, this proves to be especially difficult as re-

search and development will require substantially more time, and many challenges still need to be overcome in view of enabling manufacturing processes, which are difficult to control and handling properties, which cannot be fully characterised. Having to deal with various components for one product (e.g., specific contrast agents for molecular imaging combining nanoparticles with biological entities) will result in a dramatic increase in the expenditures in relation to the research required by these products and the legal work to support it. The large variety of sometimes overlapping definitions used in the nano-specific field does not lead to greater levels of confidence. Therefore, it is essential that the standards for toxicity tests are improved and shared between the groups specialising in the investigation of the toxicity of industrial nanoparticles (high amounts and high environmental and health risks) and pharmaceutical nanoproducts (low amounts, already regulated under the EMA). Together with the leading journals in the nanotechnology and nanomedicine fields, high standards for publications focusing on materials, processes, and methods should be put in place and the culture of scientists and editors should slightly shift to accept the "positive" outcomes of toxicity tests, which, in a sense, show no effects as well. Such measures may result in a reduction of the number of published work in this field but will, hopefully, lead to an improvement in the quality of the research conducted in this area.

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