

OECI 2008
Scientific Conference

Nanotechnology applications in cancer
prevention and treatment

Nanotechnology: going small for a giant leap in cancer diagnostics and therapeutics

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ABSTRACT

“There is *Plenty* of Room at the Bottom” - not just “There is Room at the Bottom.” What I have demonstrated is that there *is* room - that you can decrease the size of things in a practical way. I now want to show that there is *plenty* of room.

Richard Feynman, December 29, 1959

More than 30 years ago Richard Feynman pointed out that physicists knew no limits to prevent us from doing engineering at the level of atoms. Until recently, though, while the lack of physical limits was accepted as commonplace, molecular engineering was thought of as impractical, unnecessary, or requiring breakthroughs in knowledge and technique that placed it somewhere in the distant future. Many visionaries intimately familiar with the development of silicon technology still forecast it would take between 20 and 50 years before molecular engineering became a reality. This is well beyond the planning horizon of most companies. But recently, everything has begun to change. After the industrial revolution and the “computer age”, are we really facing a new *era*?

Introduction

Recent years have witnessed an unprecedented rapid growth in the area of biological sciences. In this context, the explosion of nanosciences could not have happened in a better period. Nanotechnologies represent a paradigm change in the study of and interaction with normal and cancer cells in real time and at a molecular scale. Although there is increasing optimism that nanotechnologies applied to medicine will bring significant advances in cancer diagnostics and therapy, many challenges have still to be overcome. Nanotechnology is a concept that refers to the research and technological development of different objects in a scale range of 1 to 100 nanometers. In this range, matter shows properties that are quite different from those seen in the bulk scale. Interestingly to biomedical scientists, nanotechnologies are opening new research avenues: the novel properties of nanomaterials are offering new possibilities to interact with complex biological functions operating at the very same scale of biomolecules. In these first years of the 21st century, scientist have begun to understand the unique atomic and molecular properties at this nanometer scale. Manipulation of the chemico-physical properties on this scale gives researchers the ability to build and use nanoparticles for different purposes, as drug delivery vectors, image contrast agents, and diagnostic tools.

The OEIC 2008 Scientific Conference focuses on the emerging field of nanobiotechnology and involves leading European experts. The discussions and recommendations that will be presented in Genoa should ensure continuing European cutting-edge research and development in the field of nanobiotechnology and nanomedicine whilst reducing healthcare costs.

Key words: nanotechnology, nano-medicine, drug delivery, nanoparticles

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Received;
accepted

The importance of being small

The most obvious advantage offered by nanotechnology in relation to biological systems is the ability to control and manipulate matter on a nanometer scale. Devices and components at this scale are of the same size of biological moieties (Figure 1) such as enzymes or receptors, which are much smaller than human cells and organelles. For example, human hemoglobin has a diameter of about 50 Å (5 nm), the length of a fibrinogen molecule is about 450 Å (45 nm), the diameter of a typical single wall carbon nanotube (SWCNT) can be as small as 1 nm. Nanoparticles smaller than 20 nm can easily pass through the blood-brain barrier or the blood vessel walls^{1,2}; if magnetic, they can be used, for example, to image metastatic lesions in lymph nodes³. To be considered as good drug carriers, nanoparticles must have a size smaller than 200 nm in order to be able to avoid the filtration by the spleen and to pass the liver fenestrae⁴. Such small dimensions would let a nanodevice interact with molecules on the surface of a cell, such as receptors and other signaling proteins, thus allowing the control of the regulatory mechanisms that undergo cellular changes during a disease process⁵. Despite their small size, however, nanoparticles are still able to accommodate a large number of small molecules or atoms such as drugs or the magnetic resonance imaging (MRI) contrast agent gadolinium. When attached to a dendrimer, this agent can generate a 50-fold stronger signal than in its “stand-alone” form. Given that nanoscale particles can host multiple gadolinium ions, it is obvious that such a construct would offer the opportunity to create a very powerful agent, able to

identify a tumor at a scale of 100,000 cells, far smaller than the 1,000,000,000 cells detectable with the technology available today⁶. Nanoscale MRI is already able to spot tumors and metastatic lesions at an early stage, before they are visible to the human eye. Imaging agents at the nanometer scale could be used to monitor the changes that occur in the environment surrounding a tumor site, such as the process of angiogenesis. Various MRI methods are already being directed to integrins expressed on growing capillaries, allowing to distinguish different stages of angiogenesis and thus offering the opportunity to choose the most appropriate therapeutic option to target angiogenesis.

Tumor targeting by nanoparticles

Modification of a nanoparticle's surface allows us to bind (covalently or not) a wide variety of chemical moieties: this is particularly necessary in the case of highly hydrophobic nanovectors such as carbon nanotubes (CNTs); a nanoscaled object with a high degree of hydrophobicity on its surface, when administered *in vivo*, would be taken up primarily by the reticuloendothelial system, limiting the circulation time and thus hindering its benefits. The possibility to attach hydrophilic molecules (such as polyethylene glycol chains) on the external layer of a nanoparticle greatly increases the solubility and protects the carrier from enzymatic degradation when used *in vivo*. In drug delivery, 2 modalities are employed to target nanoparticles to tumor cells: passive and active targeting. Passive targeting takes advantage of the small size of nanoparticles and the properties of tumor vasculature. In the tumoral endothelium, vessels possess wide fenestrations whose size can range between 200 nm and 1 μm⁷. In contrast to normal endothelium, these large pores allow the passage of nanoparticles of appropriate size to the extravascular spaces and the consequent accumulation inside the tumor (Figure 2A). In active targeting, tumor-specific antibodies are bound to nanoparticles, giving them the ability to target specific cell surface proteins (Figure 2B). Several targeting molecules have been successfully used including folate⁸, thiamine¹, aptamers⁹, and a wide variety of monoclonal antibodies directed to cell-surface proteins such as integrins¹⁰. In the active targeting process, once bound to the target cell, nanoparticles are internalized by receptor-mediated endocytosis^{8,11,12}.

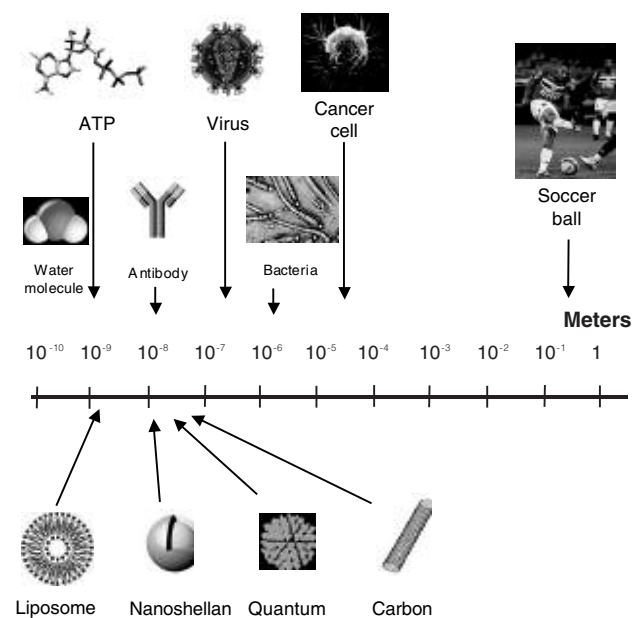


Figure 1 - Relative size of nanoparticles.

Drug carriers

Nanosized drug delivery systems have already entered clinical use and Europe has been pioneering this discipline. The application of nanotechnology to design materials able to target specific cells or pass across different biological barriers is a challenge of modern med-

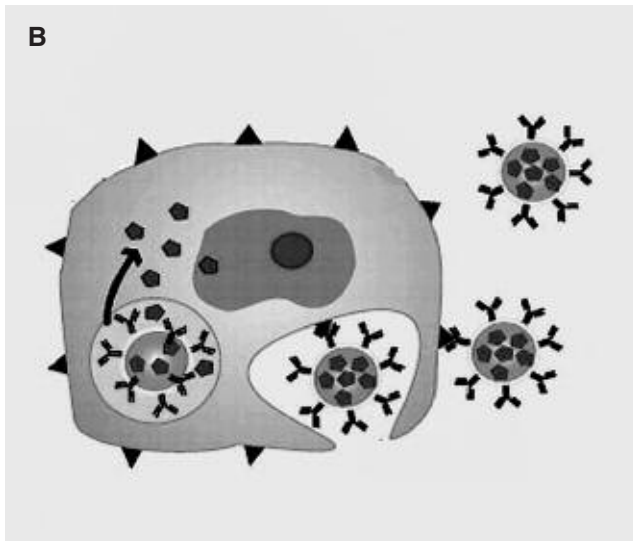
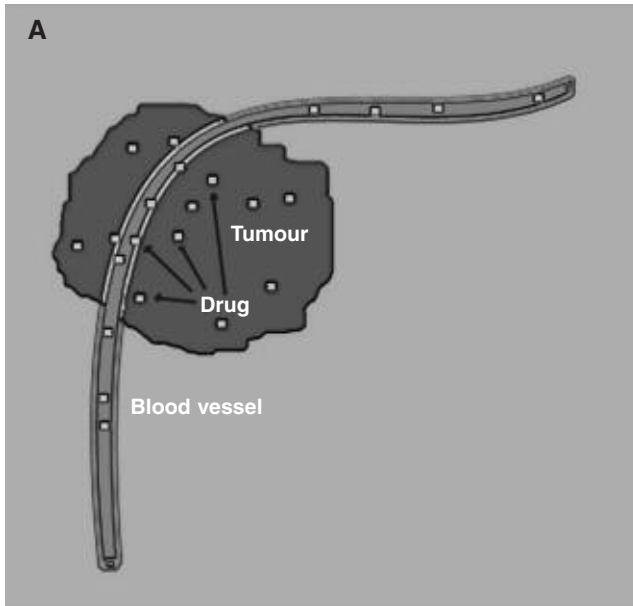


Figure 2A - The enhanced permeability and retention (EPR) effect.
Figure 2B - Active targeting of functionalized nanocapsules.

icine. A number of possible carriers are currently being studied (Figure 3).

Colloidal drug carrier systems are encapsulated or dispersed systems which typically have a diameter of 10-400 nm. Their properties make these micellar systems very promising¹³. High-molecular-weight micellar systems are formed by self-assembly of short amphiphilic block copolymers (5-50 nm) in aqueous solutions. Due to their structure, they are able to accumulate in solid tumors, using the enhanced permeability and retention (EPR) effect. The active molecules can be entrapped in the core of micelles and transported even at concentrations that can exceed their intrinsic water sol-

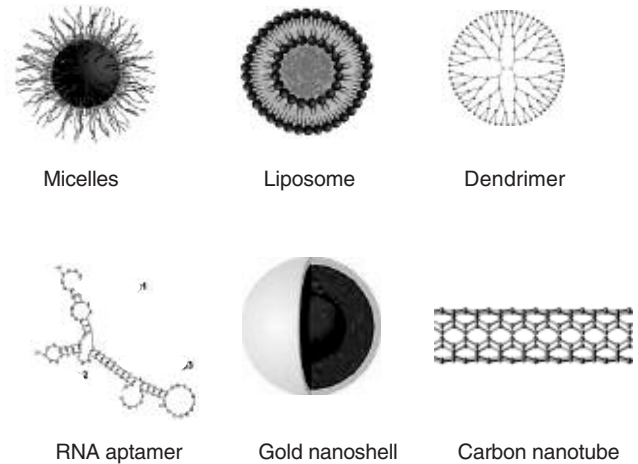


Figure 3 - Different examples of pharmaceutical carriers.

ubility¹⁴. Moreover, micelles can effectively protect drugs (proteins, nucleic acids or polysaccharides) from external hydrolysis and enzymatic degradation.

Liposomes are a form of phospholipid vesicles consisting of different or just one phospholipid bilayer. Their diameter size ranges between 50 and 250 nm, thus enabling polar drug molecules to be encapsulated in their core. These carriers display interesting properties such as the ability to use the EPR effect to accumulate drugs in solid tumors. Amphiphilic and lipophilic molecules could be solubilized within the phospholipid bilayer. In this way channel proteins could be incorporated into a liposome shell without losing their activity. Acting as a size-selective filter, these proteins could allow passive diffusion of small solutes such as ions, protecting the drugs from degradation by proteolytic enzymes. At the same time, the drug molecule is able to diffuse through the channel, driven by the different concentration between the core and the exterior of the liposome. While at first the liposomes showed high instability under physiological conditions resulting in total release of the internal content (burst effect), the stability improved thanks to the introduction of multi-vesicular liposomes.

Dendrimers are repeated, highly branched molecules characterized by their symmetry and monodispersity. They consist of a central core, different symmetrical branching units and functional groups on the molecular surface. The most interesting aspect of dendrimers is that their size and composition can be easily controlled by pH, temperature and concentration. The internal core can be so designed as to determine the environment of the cage occupied by the drug, whereas the external groups determine the solubility and chemical behavior of these polymers¹⁶. Dendrimers may also be built around a template that can act as the core. It is then possible to cross-link the structure and remove the

template, creating a structure at the core of the dendrimer that has a specific binding site with the ability to separate enantiomers. In addition, one can easily entrap metal ions within dendrimers, thus creating fluorescent nanoparticles. By using a low concentration of metal-dendrimer composite in order to minimize toxicity, it would then be possible to perform intracellular fluorescent assays¹⁷.

Nanoparticles are in the solid state and can be either amorphous or crystalline. They are able to encapsulate a drug, thus protecting it against enzymatic degradation. Nanocapsules are vesicular systems consisting of a shell and an empty core in which the drug is confined, while nanospheres are matrix systems, presenting a uniform cross-section in which the drug is physically and uniformly dispersed. The most studied nanoparticles for drug delivery are metallic nanoshells made of a dielectric core and covered by a thin layer of metal. The particular composition of metallic nanoshells (e.g., gold-silica nanoshells) gives them unique physical properties such as light absorption in the near infrared region, where the absorption of biological matter is low. Once the particle has reached the tumor site, external radiation can exit the nanoshell and destroy the tumor by local thermal heating.

Hydrogels are 3-dimensional, hydrophilic polymer networks (i.e., collagen, gelatin, dextrans etc.). They can swell and thus change their diffusion properties when exposed to water or biological fluids. The networks can be composed of homopolymers or copolymers. The presence of chemical cross-links (tie-points, junctions) makes them insoluble. Hydrogels are used to regulate drug release in the presence of a reservoir. **It is possible to build hydrogels that are sensitive to an applied charge, to particular enzymes or antigens, or to pH changes, so that drug release can be programmed to occur within specific areas of the body or via specific sites.** [AUTHORS: Change OK?]

Covalent conjugation of biological (peptides/proteins) and synthetic polymers is another efficient way of drug delivery. The modification of proteins relies on the particularity of the functional groups of some amino acid residues such as lysines, cysteines, glutamic and aspartic acid, and tyrosines. The use of a polymer bioconjugated to an active peptide or protein can reduce toxicity, also preventing immunogenic or antigenic side effects. It is possible to use cavities to host other elements like covalently bound small molecules. Recently the heat-shock protein has been modified by introducing cysteine residues on the protein surface and then selectively attaching an antitumoral drug, doxorubicin, to the thiol groups of these residues using a pH-sensitive linker. The modified protein assembled into a 24-subunit structure with a diameter of about 12 nm allows the exchange of small molecules between the interior and the bulk solvent solution^{18,19}.

Applications in imaging and early detection

Nanoparticle applications are not limited to the body: indeed, one of the most promising fields of application of nanotechnology is the development of tools at the nanometer scale for *in vitro* diagnostics. Nanocantilevers coated with antigens are studied for use as detectors of antibodies and functionalized CNTs could be used also as highly specific biosensors²⁰. Another area with near-term applicability is the detection of mutations and genome instability *in situ*. Nanopores are being investigated as potential “real-time” DNA sequencers, making it possible to distinguish among different types of tumors quickly and accurately. Nanosensors could also be used to detect environmental and/or lifestyle cancer risk factors; these tools will be useful not only to identify subjects at risk of developing cancer, but also to start new studies on gene-environment interactions and on the relationship between these interactions and the development of (or the resistance against) cancer.

Besides the other applications of different nanoparticles (gold nanoshells, quantum dots, CNTs), a discussion about the *in vitro* application of nanotechnology would not be complete without an overview of the instruments used to build these entities. Traditional spectroscopy (FTIR and Raman) together with electron microscopy are used to characterize the functionality, purity and weight of molecules; atomic force microscopy (AFM) is the technique that mostly influenced the advent of the nanotechnology era. The capability and accurate sensitivity of AFM derive from the use of a nanoscaled probe attached to a cantilever which is able to sense and measure the interatomic interactions with the substrate. The spatial resolution of AFM is a few ångström (1Å = 0.1 nm) and nowadays it is widely used in the biomedical field to elucidate biomolecular structures in conditions very close to physiological, measuring forces in bindings (i.e., receptors and ligands, antibody and antigens), observing the topological surface of viruses, and imaging histological features.

Some concerns

We have illustrated how nanotechnologies offer many benefits and will continue to do so in the future; however, they may affect our lives in a way that may not be the one researchers are aiming at. A public debate is needed about their development. In particular, there is a strong need to improve the understanding of the toxicological implications of nanomedicine in relation to the specific properties of nanomaterials. Consideration should be given to the environmental impact and to a safety assessment of the whole manufacturing process. A risk-benefit assessment is needed in respect to both acute and chronic effects of nanomedicine application for medical purposes.

The toxicity of nanoparticles seems to be linked to their surface and not to the mass. Available studies showed different effects on animals, depending on the type of nanoparticles. In particular nephrotoxicity, effects on reproduction, granulomas, fibrosis and tumors have been observed. However, the toxicological data specific to nanoparticles are insufficient due to the small number of studies and to the short exposure period. Additional studies are necessary to assess the risk associated with inhalation and cutaneous exposure to nanoparticles.

Nanobiotechnologies: a business?

Even though the science at the basis of the physico-chemical properties of nanotechnologies is poorly understood if not mysterious, the commercial potential of the infinitesimally small is coming sharply into focus. In recent years, large and small companies have introduced more products from the lab into the market. People are already using nanotechnologies in several goods: car manufacturers employ nanoceramics in the construction of stronger and lighter turbines, advanced paints and chassis, aeronautics will extensively use CNTs in the next generation of hypersonic airplanes; even golf balls designed to go straight will be built using nanomaterials. Computer industries are planning within 10 years to revolutionize the technology of computing by improving the power of silicon-based machines. The nanopharma industry of the future is late to arrive but, as shown by experience, we need to invest today if we want to be present as leading actors in the nanoworld of personalized medicine.

Europe is currently among the world leaders in nanotechnology but nanotechnology science in the European Union needs a sustainable environment for research in order to remain globally competitive and realize its international potential. Securing future prosperity and exploiting the value of the technology requires the development of a specific government funding strategy, with a central body to coordinate research initiatives. This will minimize redundancy in research and facilitate the development of technology that addresses the most urgent socioeconomic needs of the planet. Establishment of good communication is a universal challenge for research and development, particularly for emerging technologies. There is a need to promote transdisciplinary conferences and research partnerships between large medical centers and universities. Moreover, it is mandatory to make serious efforts to ensure that politicians are well briefed in the topic: better diagnostics, treatment and prevention will bring healthcare benefits. Research and development in nanomedicine will also offer employment and economic benefits with a parallel reduction of healthcare budgets.

Conclusions

It is generally felt that the technological basis of nanotechnology is already developed enough to enable physicians and biologists to make ready use of these materials. This is not completely true: there are still a lot of fundamental questions concerning these materials that have to be answered. Standardization assays need to be developed and risks should be carefully evaluated. The integration of nanotechnology with cancer research and diagnostics is a rapidly advancing field and there is an urgent need to understand the concepts arising from this new technology.

The OECI meeting in Genoa will define the remit of the emerging field of nanotechnology in oncology. This field is clearly multidisciplinary and builds on expertise in a wide range of scientific areas, from physics to colloidal chemistry, from molecular biology to membrane biophysics, from medicine to cell physiology. The strengths of the European Union have been clearly identified in terms of short- and long-term opportunities. An open dialogue has been launched to safeguard all interested parties, including industries and the general public. It is now important to clearly distinguish what is science from what is science fiction.

References

1. Lockman PR, Oyewumi MO, Koziara JM, Roder KE, Mumper RJ, Allen DD: Brain uptake of thiamine-coated nanoparticles. *J Control Release*, 93: 271-282, 2003.
2. Vinogradov SV, Batrakova EV, Kabanov AV: Nanogels for oligonucleotide delivery to the brain. *Bioconjug Chem*, 15: 50-60, 2004.
3. Bogdanov AA Jr, Chen JW, Kang HW, Weissleder R: Magnetic resonance signal amplification probes. *Ernst Schering Res Found Workshop*, 49: 147-157, 2005.
4. Moghimi SM, Hunter AC, Murray JC: Long-circulating and target-specific nanoparticles: theory to practice. *Pharmacol Rev*, 53: 283-318, 2001.
5. Hanahan, D, Weinberg RA: The hallmarks of cancer. *Cell*, 100: 57-70, 2000.
6. Morawski AM, Winter PM, Crowder KC, Caruthers SD, Fuhrhop RW, Scott MJ, Robertson JD, Abendschein DR, Lanza GM, Wickline SA: Targeted nanoparticles for quantitative imaging of sparse molecular epitopes with MRI. *Magn Reson Med*, 51: 480-486, 2004.
7. Hobbs SK, Monsky WL, Yuan F, Roberts WG, Griffith L, Torchilin VP, Jain RK: Regulation of transport pathways in tumor vessels: role of tumor type and microenvironment. *Proc Natl Acad Sci U S A*, 95: 4607-4612, 1998.
8. Quintana A, Raczka E, Piehler L, Lee I, Myc A, Majoros I, Patri AK, Thomas T, Mule J, Baker JR Jr: Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor. *Pharm Res*, 19: 1310-1316, 2002.
9. Farokhzad OC, Jon S, Khademhosseini A, Tran TN, Lavan DA, Langer R: Nanoparticle-aptamer bioconjugates: a new approach for targeting prostate cancer cells. *Cancer Res*, 64: 7668-7672, 2004.
10. Li L, Wartchow CA, Danthi SN, Shen Z, Dechene N, Pease J, Choi HS, Doede T, Chu P, Ning S, Lee DY, Bednarski MD,

- Knox SJ: A novel antiangiogenesis therapy using an integrin antagonist or anti-Flk-1 antibody-coated ⁹⁰Y-labeled nanoparticles. *Int J Radiat Oncol Biol Phys*, 58: 1215-1227, 2004.
11. Kreuter J: Influence of the surface properties on nanoparticle-mediated transport of drugs to the brain. *J Nanosci Nanotechnol*, 4: 484-488, 2004.
 12. Oyewumi MO, Mumper RJ: Engineering tumor-targeted gadolinium hexanedione nanoparticles for potential application in neutron capture therapy. *Bioconjug Chem*, 13: 1328-1335, 2002.
 13. Storm G, Crommelin DJ: Colloidal systems for tumor targeting. *Hybridoma*, 16: 119-125, 1997.
 14. Yokoyama M: Block copolymers as drug carriers. *Crit Rev Ther Drug Carrier Syst*, 9: 213-248, 1992.
 15. Zhao Y: Rational design of light-controllable polymer micelles. *Chem Rec*, 7: 286-294, 2007.
 16. Patri AK, Majoros IJ, Baker JR: Dendritic polymer macro-molecular carriers for drug delivery. *Curr Opin Chem Biol*, 6: 466-471, 2002.
 17. Lesniak W, Bielinska AU, Sun K, Janczak KW, Shi X, Baker JR Jr, Balogh LP: Silver/dendrimer nanocomposites as biomarkers: fabrication, characterization, in vitro toxicity, and intracellular detection. *Nano Lett*, 5: 2123-2130, 2005.
 18. Flenniken ML, Willits DA, Harmsen AL, Liepold LO, Harmsen AG, Young MJ, Douglas T: Melanoma and lymphocyte cell-specific targeting incorporated into a heat shock protein cage architecture. *Chem Biol*, 13: 161-170, 2006.
 19. Flenniken ML, Liepold LO, Crowley BE, Willits DA, Young MJ, Douglas T: Selective attachment and release of a chemotherapeutic agent from the interior of a protein cage architecture. *Chem Commun*, 28: 447-449, 2005.
 20. Chen RJ, Bangsaruntip S, Drouvalakis KA, Kam NW, Shim M, Li Y, Kim W, Utz PJ, Dai H: Noncovalent functionalization of carbon nanotubes for highly specific electronic biosensors. *Proc Natl Acad Sci U S A*, 100: 4984-4989, 2003.