ADVANCES IN NANOMEDICINE: OPPORTUNITIES AND CHALLENGES
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A. Introduction

The term ‘Nanotechnology’ was first defined in the year 1974 by Norio Taniguchi of the Tokyo Science University. According to Professor Taniguchi’s definition, “Nanotechnology mainly consists of processing, separation, consolidation, and deformation of materials by one atom or by one molecule.” The National Nanotechnology Initiative (NNI), defines Nanotechnology as “…the science, engineering and technology related to the understanding and control of matter at the length scale of approximately 1 to 100 nanometers …also research and development of materials, devices, and systems that have novel properties and functions due to their nanoscale dimensions or components.” Nanomedicine is a subset of nanotechnology as applied to medicine i.e. diagnosis, treatment, monitoring and control of disease and biological processes. There are numerous products which are being developed or are currently on the market making use of nanotechnology such as nanoparticles, nanorods, nanopores, quantum dots and nanoshells. Nanotechnology in the field of medicine is being used for a number of applications ranging from drug delivery to diagnosis and imaging. 1, 2, 3, 4

The purpose of this review article is to highlight advances in the field of nanotechnology as applied to Nanomedicine and discuss various systems in clinical and preclinical studies. Opportunities in disease diagnosis and therapy will be presented along with a discussion on key product development challenges and regulatory initiatives.

B. Marketed Systems and in Clinical Development

Nanomedicine related products have been on the market for over 17 years now and constitute a large industry, for example Nucryst Pharmaceutical’s Acticoat™ antimicrobial barrier dressings with SILCRYST™ nanocrystals was first introduced in 1998 and is currently marketed in over 30 countries. It is one of the fastest growing markets and its revenues are expected to reach multi-billion dollars by year 2011. Other products in this category currently on the market and in development are discussed below: 5, 6

1. Gold Nanoparticles for diagnostic and therapeutic applications: Gold nanoparticles in the form of nanorods, spheres and nanowires are being used in the products by Nanopartz for diagnostics, imaging and therapeutics. For example, Ntracker™, Ntherapy™ and Nsense™ nanorods are intended to be used for photoacoustic imaging, optical coherence tomography (OCT), two photon fluorescence and in vivo SERS imaging. These have been found to be devoid of in vivo cytotoxicity and long circulation times in vivo.

Ntherapy™ nanorods having a coating of hydrophilic polymers with varied terminations viz. amine, carboxyl, and neutravidin is being used for drug delivery. Ntherapy™ has long circulation times in vivo owing to the hydrophilic polymer coating and it has the advantage of conjugating with a number of targets due to the varied terminations.

Ntherapy™ and Ntracker™ nanorods have an axial diameter of 10 nm and length between 29-45 nm, while Nsense™ nanorods have an axial diameter of 25 nm and length varying between 35-86 nm.

Other than that, various products for in vitro diagnostics and imaging are also provided by Nanopartz™ viz. Gold nanobeads™ (Diameter: 5-100 nm), Accurate™ Spherical gold nanoparticles (Diameter: 5-100 nm), Microgold™ (Diameter: 150 or 200 nm and length: 800 or 1000 nm), Accurate™ Conjugated spherical gold nanoparticles (Diameter: 5-100 nm) etc.

The Gold nanobeads™ are gold colloid containing polymer-caged nanoparticles with varied terminal groups such as methyl, carboxyl, amine, biotin or neutravidin. These beads are stable at pH ranging from 3 to 11 and have a shelf life up to one year. The Accurate™ Spherical gold nanoparticles are available in unconjugated as well as conjugated form. The Accurate™ conjugated form includes nanoparticles conjugated with functional groups such as carboxyl, methyl, amine, biotin, or neutravidin. These have the advantage of conjugating with most antibodies and DNA and are particularly useful for in vivo applications. Microgold™ on the other hand is the largest gold nanomaterial available and is used for a variety of purposes such as labeling in electron microscopy and as sensors etc.7

Colloidal gold nanoparticles for targeted delivery of drugs to tumors are being developed by Cytimmune Sciences Inc. Their product Aurmine™ (CYT6091, Diameter: 27 nm), pegylated colloidal gold nanoparticle with attached tumor killing agent, recombinant human tumor necrosis factor alpha (TNF), was found to be safe and effective in Phase I clinical trials. Aurmine™ will undergo Phase II clinical trials soon and is expected to hit the markets after FDA regulatory approval. Another product by
Cytilimmune Sciences Inc. is AuriTol™ (CYT21001), a pegylated colloidal gold nanoparticle with an additional chemotherapy drug, paclitaxel for improved efficacy. AuriTol™ has completed preclinical studies and is expected to enter Phase I clinical trials soon. Other products in Cytilimmune’s pipeline are Pegylated Colloidal Gold bound TNF with Paclitaxel; CYT-31000, (AuriCin™) Pegylated Colloidal Gold bound TNF with Doxorubicin; CYT-41000, Pegylated Colloidal Gold bound TNF with Interleukin-12; CYT-51000, Pegylated Colloidal Gold bound TNF with Interleukin-2.

2. **Nanocochleates for oral administration:** BioDelivery Sciences International Inc., has come up with a technique of encapsulating a drug in a nanocrystalline structure called nanocochleate (Bioral™) for oral drug delivery. The nanocochleates having a diameter of 50 nm are the crystalline structures with anhydrous interior that encapsulates the drug molecule and protects it from degradation in gastrointestinal tract. The first product employing this technique is Bioral™ Amphotericin B.

Bioral™ Amphotericin B underwent Phase I clinical trials for evaluation of its safety, tolerability and pharmacokinetics. It was found to be well tolerated in healthy human volunteers and the initial pharmacokinetic studies revealed a measurable concentration of drug in the tissues. It is soon expected to undergo Phase II clinical trials after additional pharmacokinetic studies in healthy human volunteers.

3. **Antimicrobial Nanoemulsions:** NanoBio corporation’s NanoStat™ technology based products are nanoscale oil-in-water emulsions intended to be used topically as well as in the form of mucosal vaccines. This technology has proven to be effective against a number of bacteria, viruses, fungi and spores. The nanosized droplets of emulsion disrupt the outer membrane of pathogenic organisms thereby killing them. There are numerous mucosal (intranasal) vaccines in the NanoBio’s pipeline for diseases like seasonal influenza, hepatitis B, HIV, pneumococcal, smallpox, RSV, anthrax, and cancer. NB-001 for the treatment of cold sores is licensed to GSK Consumer Healthcare based on a press release in December of 2009 and is being advanced to Phase 3 clinical trials.

NanoBio’s seasonal influenza intranasal vaccine NB-1008 underwent Phase-I clinical trials in April 2009 to prove its safety and immunogenicity. The vaccines for Hepatitis B, Anthrax, Small pox and trivalent (Anthrax/Plague/Botulinum) are in the preclinical phase and those for Pandemic influenza, Pneumococcal, RSV and cancer are in the discovery phase.

4. **NanoXray:** Nanobiotix’s NanoXray™ is a solution to the problems posed by existing cancer therapies viz. destruction of healthy cells and tissues along with the tumor cells. NanoXray™ is a nanoparticle (named Nbtx3 ) having an inactive and inert core coated with biocompatible surface active agent. The nanoparticles effectively penetrate the tumor cells and emit electrons upon exposure to X-rays. This results in the formation of reactive free radicals which damage membranes, proteins and nuleic acid and subsequently kill the tumor cells.

Preclinical studies of Nbtx3 have shown uptake of nanoparticles by mammalian tumor cells, improved performance as seen in cell viability assays, and longer residency time of nanoparticles in tumors for at least 15 days with a good dispersion of the product in tumor site. It is currently undergoing Phase I clinical trials.

The products in Nanobiotix’s pipeline using nanotechnology are nanoMag™ and nanoPDT™. NanoMag™ uses magnetic particles for the treatment and diagnosis of cancer and nanoPDT™ employs laser activation of nanoparticles for cure of cancer.

C. **Systems in Preclinical Development.**

1. **Delivery of antibiotics via nanoparticles:** Aerosolized nanoparticles for the delivery of antibiotics to the lungs may prove beneficial for the treatment of respiratory diseases. This delivery mechanism is intended for controlled release of drugs so as to decrease the number of doses required and thereby increase patient compliance. This has the added advantage of reduced side effects and emergence of resistant bacterial strains associated with high and frequent dosing of antibiotics.

The recent work in this area is done by Carolyn L. Cannon from Washington University School of medicine in collaboration with Center for Silver Therapeutics Research at the University of Akron. They illustrated that the aerosolized nanoparticles encapsulated silver-based antibiotics, such as silver carbene complexes, treated mice infected with a common bacterial species, Pseudomonas aeruginosa with a significant survival advantage over controlled mice. This also resulted in decreased bacterial load in the lungs. Toxicity studies need to be performed for this delivery mechanism to undergo clinical trials and eventually be commercialized.

2. **Nanoparticle-mediated gene transfer stimulating neurogenesis:** Nanoparticles for the first time are being employed for the delivery of novel Integrative FGFR1 Signaling (INFS) that stimulates neurogenesis in brain by researchers at the University of Buffalo (UB). According to Michal Stachowiak, Ph.D., director of the Molecular and Structural Neurobiology and Gene Therapy Program at UB, nanoparticle mediated non-viral gene delivery is a safer alternative to viral gene delivery. It can be used to manipulate the neural stem cells to differentiate so as to repopulate the degenerated brain areas. This can particularly prove...
to be beneficial in neurodegenerative disorders such as Alzheimer’s disease, Parkinson’s disease and neurodevelopmental disorders like autism and schizophrenia.19

3. **Nano implantable device for cancer monitoring:** Michael Cima, MIT professor of materials science and engineering and colleagues have developed a nanodevice that can help in the diagnosis of cancer. These tiny devices are 5 millimeter long cylindrical implants made of polyethylene containing magnetic particles covered with antibodies specific to target molecule and coated with a semi-permeable membrane. The target molecules if present in the body, enter the device through semi-permeable membrane and bind to the antibodies resulting in clumping which can further be detected using magnetic resonance imaging (MRI).20

These nanodevices can also be used to track tumor growth and monitor the effectiveness of chemotherapy agents based on the response of tumor to the treatment viz. whether it is growing or shrinking.

4. **Nanorods for diagnosis and treatment of cancer:** Another latest advancement in the area of nanotechnology is the discovery of tiny gold particles called gold nanorods. Geoffrey von Maltzahn, MIT graduate student developed these gold nanoparticles which can accumulate in the tumors due to the relatively large pore size of blood vessels in the tumors when injected in the blood stream. When treated with near-infrared laser, the nanorods will heat up and kill the tumor cells.

These particles can also be used for diagnosis of cancer since they can be imaged employing Raman scattering. Also, if less heat is applied these nanoparticles can also be used as a supplement to the existing chemotherapy treatments.

These nanoparticles depending on their shape can absorb light of different frequencies, for example rod shaped particles absorb light at near-infrared frequency. 21

5. **Nano gold particles as drug delivery system:** MIT researchers have come up with a new technique employing nano gold particles as drug payloads. These particles release drugs from their surface as they melt under the influence of infrared light. Using this technique multiple drugs can be released in a controlled fashion and can particularly be useful for the treatment of diseases requiring more than one drug. The release timing can be controlled using the external exposure to infrared light.

The release from these nanoparticles can also be controlled using different infrared wavelengths as different shape of nanoparticles respond to different wavelength. Two types of particles have been synthesized viz. nanobones and nanocapsules, both of which melt at different wavelengths i.e. 1100 nm and 800 nm respectively. And it is estimated that up to four different shaped nanoparticles can be developed releasing drug payload at different wavelengths.22

6. **Quantum Dots for Cell Imaging:** Nanosized quantum dots have been identified by Scientists at UCLA as a tool for visualizing and mapping protein binding sites on DNA. This technique can help in understanding cellular processes like gene expression and regulation and may prove beneficial for genetic analysis.

Another fluorescent sensor called Histac™ is developed by Minoru Yoshida and Kazuki Sasaki of the RIKEN Advanced Science Institute in Wako. Histac™ is a sensitive genetically encoded indicator that helps in real-time imaging of changes in H4 acetylation. Histone proteins are responsible for the coiled and compact form of DNA called chromatin. Any change in the histones can alter the arrangement of chromatin and this might affect gene expression by the cells.23

**Challenges:** In spite of tremendous growth, increasing revenues and the impact nanomedicine has made on healthcare, there exist key product development challenges which need to be proactively considered and addressed. These include, processing and manufacturing, stability, pharmacokinetics, safety/toxicity and environmental issues. The toxicity and environmental concerns associated with nanomedicine products is due at least in part to their extremely small size, and surface charge and composition. These properties provide opportunities for increased uptake and interaction with biological tissues relative to bulk materials. Potential nanomaterial exposure routes are inhalation, dermal contact and ingestion. Upon inhalation, nanomaterials can cause lung injury and other complications. Nanomaterial manufacturing can also generate toxic intermediates and byproducts that pollute the environment.

Thus, it becomes necessary to assess the risk-benefit ratio of nanomedicines and this calls for strict regulations for nanotechnology based products in general. There is a need for guidelines and standards for the manufacturing processes, quality assessment and characterization of products employing nanotechnology.

FDA has taken initiative in this respect and has created a Nanotech task force to address regulatory and scientific issues. The task force was formed by Commissioner Andrew C. von Eschenbach of U.S. Food and Drug Administration (FDA) in 2006 with a mission to define the regulatory approaches so as to encourage the development of safe and effective products and therapies employing nanotechnology. On July 25, 2007, a report by FDA’s nanotechnology task force recommended the development of guidelines for drug products and medical devices using nanotechnology which is underway. FDA has also formed a NanoTechnology Interest Group (NTIG) to assist in the regulation of nanomedical products.
Currently, the European Union has no specific regulations for nanomedicines and the nanomedical products are being regulated under existing legislations.

The major hindrance in the development of regulations for nanomedicines is vague borderline between product and device. Nanomedicines are often combination products viz. drug-device, drug-biologic, device-biologic product. Also, another issue is insufficient knowledge regarding characterization, assessment of safety and efficacy of nanomedicines. There is clearly a need to determine what lessons learned from the marketed nanomedicine products can be applied to products in development and what is truly new science and knowledge. 24, 25, 26

Conclusion: Nanotechnology as applied to 1-100 nm materials, devices and systems is an emerging technology with a promising future in the arena of healthcare. It has proven to be beneficial in various aspects but also posed certain potential risks. Hence, there is a need of sound regulatory framework for innovative products making use of nanotechnology. This can be achieved with the participation and consultation of various regulatory authorities worldwide and research institutes from industry and academia. Drug discovery, delivery and development applications of nanotechnology is also the scope of the AAPS Nanotechnology Focus Group with global, academic, government and industrial members and which is affiliated with the FDD and PPB Sections of the AAPS. It is an excellent forum to discuss and address the various areas presented in this nanomedicine review article.

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References:

1. http://www.nanomedicine.com/NMI/1.3.1.htm
17. http://www.nanobiotix.com/technology-products/#otherDVP