Review Article

Nanobiotechnology: An overview of drug discovery, delivery and development

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ABSTRACT

Nanobiotechnology is a new technology concerned specifically with the functionalism and modification of chemical-physical structures on a biomolecular scale, and also is the application of nanotechnology to the life sciences. Nanotechnology for biotechnology and pharmaceutical applications has progressed from the concept stage to commercialization. Nanobiotechnology represents the future of medicine and healthcare. Various physical, chemical, electrical tools and methods used to investigate biological nanoobjects include optical tools, nanoforce and imaging, surface methods, mass spectrometry and microfludics. Its application has an impact on diagnostics, drug delivery as well as drug discovery. Nanobiotechnology focuses on various areas such as nanobiotechnology and cancer, drug discovery and tools, and nanobiotechnology and medicine. Applications are emerging from all branches of nanobiotechnology in medicine and pharmacy. Several technologies including nanoparticles and nanodevices such as nanobiosensors and nanobiochips have been used to improve drug discovery and development. Some nanosubstances such as fullerenes and dendrimers/biodendrimers could be potential drugs for the future. Moreover, nanobiotechnology has the potential for combining drug design and drug delivery. However, limitations of the available nanoparticles still to be resolved for their application in the drug-discovery studies exist. The benefits of nanotechnology are enormous and so these benefits should be maximized while efforts are made to reduce the risks.

Keywords: Nanobiotechnology, nanobiomaterials, nanobiostructures, nanocarriers

INTRODUCTION

Nanotechnology is a broader term applicable to small things in nanometer range (roughly in the 1−100 nm size regime in at least one dimension). Nanobiotechnology is a big word made up of three parts: NANO is really, really tiny, BIO is living things, and TECHNOLOGY is about tools. Nanobiotechnology is an emerging area of science which is concerned with the application of tools and processes in order to assemble devices for the study of objects in biological systems. Nanobiotechnology is that branch of nanotechnology that deals with biological and biochemical applications or uses. Nanobiotechnology often studies existing elements of living organisms and nature to fabricate new nano-devices. Generally, nanobiotechnology refers to the use of nanotechnology to further the goals of biotechnology. Relationship of nanotechnology, biotechnology and nanomedicine is shown in Figure 1.

Further, nanobiotechnology (an integration of physical sciences, molecular engineering, biology, chemistry and biotechnology) has yielded healthcare discoveries that have been used for drug delivery and diagnostic purposes.[1]

In medicine and pharmacology, nanobiotechnology opens up new perspectives in analytics and therapy. Medical applications of nanobiotechnology resulted in appearance of new field i.e. nanomedicine. An increasing use of nanobiotechnology by the pharmaceutical industries includes (i) drug delivery, and (ii) disease therapy. The potential topics in nanobitechnology

Received Date : 17-11-2011
Revised Date : 04-01-2012
Accepted Date : 17-02-2012

DOI: 10.5530/rjps.2012.1.3

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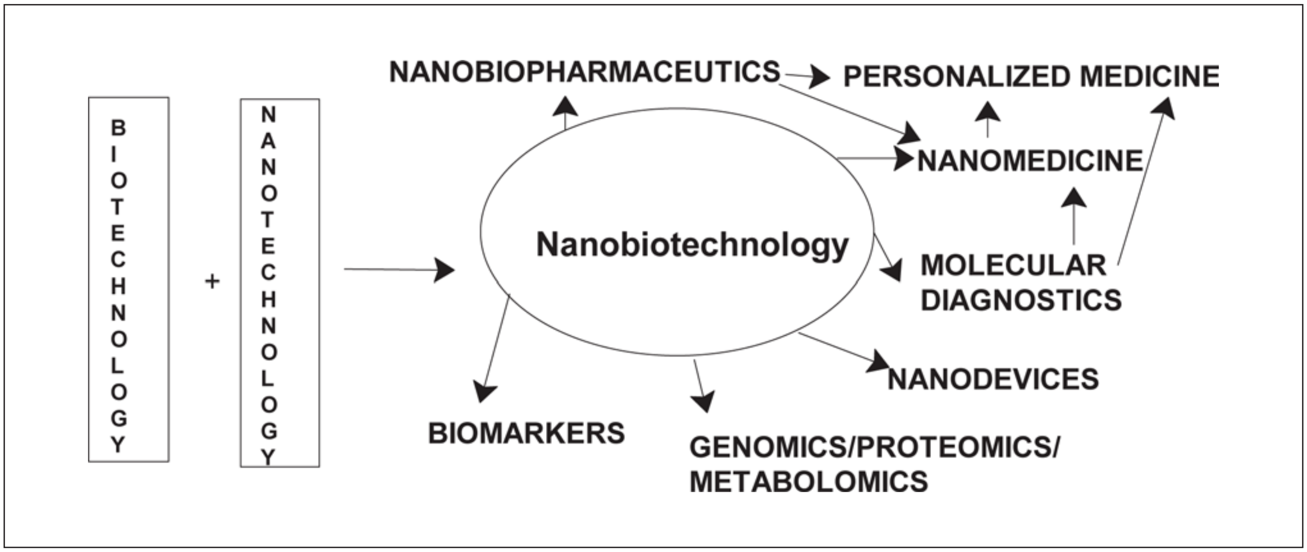

Figure 1: Relationship of nanotechnology, biotechnology and nanomedicine.

include molecular bioprobes, nanoparticles and nanobiosystems, nanobiomaterials, biomolecular assemblies and supra-biomolecules, nanobiosensors and nanobiochips, BioNEMS and nano-biofluidics, nanobiophotonics, single-molecule detection and manipulation and molecular motors. Experts are of the opinion that nanobiotechnology has the potential to yield a scientific and industrial revolution, as envisioned by the numerous programs on nanotechnology/nanobiotechnology launched over the last decade by councils and governments worldwide.

The potential uses and benefits of nanobiotechnology are enormous.[2] Nano-systems in biology, the most complex and highly functional nanoscale materials and machines have been invented by nature. Proteins and nucleic acids, and other naturally occurring molecules (polymers) regulate and control biological systems with incredible precision. Many nanotechnologists are in fact drawing inspiration from biology to device new materials and devices. Moreover, nanotechnology/nanobiotechnology is expected to rule tomorrow's world.

NANOBIOSTRUCTURES

The use of biological principles is becoming widespread in the design of nanomaterials. Representative examples of nanobiostructures include DNA nanostructure, peptide structure and biomimetics. 3D-DNA nanostructures have emerged as promising tools for biology and materials science. In this regard, DNA cages, nanotubes, dendritic networks, and crystals with deliberate variation of their size, shape, persistence length, and porosities exhibited dynamic character, allowing their selective switching with external stimuli. Short peptides can spontaneously associate to form nanotubes, nanospheres, nanofibrils, nanotapes, and other ordered structures at the nanoscale. Further, peptides can also form macroscopic assemblies such as hydrogels with nanoscale order. The theory and the mechanisms behind peptide self-assembly process and their bionanotechnology applications have been reported.[3] In general, nanobiotechnology requires the organization of atoms and molecules in a two- or three-dimensional space. Self-assembly properties of biomolecules have ability to spontaneously organize into nanostructures, which allows mimicking the living cell membranes.

Nanoparticle usually forms the core of nanobiomaterial. Nanobiomaterial is made of nanoparticles and nanobiomaterials are emerging as the most hopeful area of research within the area of biological materials science and engineering. They have an increased number of atoms and crystal grains at their surfaces and possess a higher surface area to volume ratio than conventional microscale biomaterials. These differences in surface topography alter the corresponding surface energy for protein adsorption. Nanobiomaterials can be used for human implant, orthopedics, drug delivery, gene therapy, antimicrobial treatments, array technologies, and diagnostics. Moreover, nanobiomaterials help with targeting, measuring, sensing, and imaging. The approaches used in constructing nanobiomaterials are given in Figure 2.

NANOBIOTECHNOLOGY BASED DRUG DELIVERY/ DEVELOPMENT

Nanomedical approaches to drug delivery center on developing nanoscale particles or molecules to improve the bioavailability of a drug. Nanomaterials and nanoparticles are likely to be cornerstone of

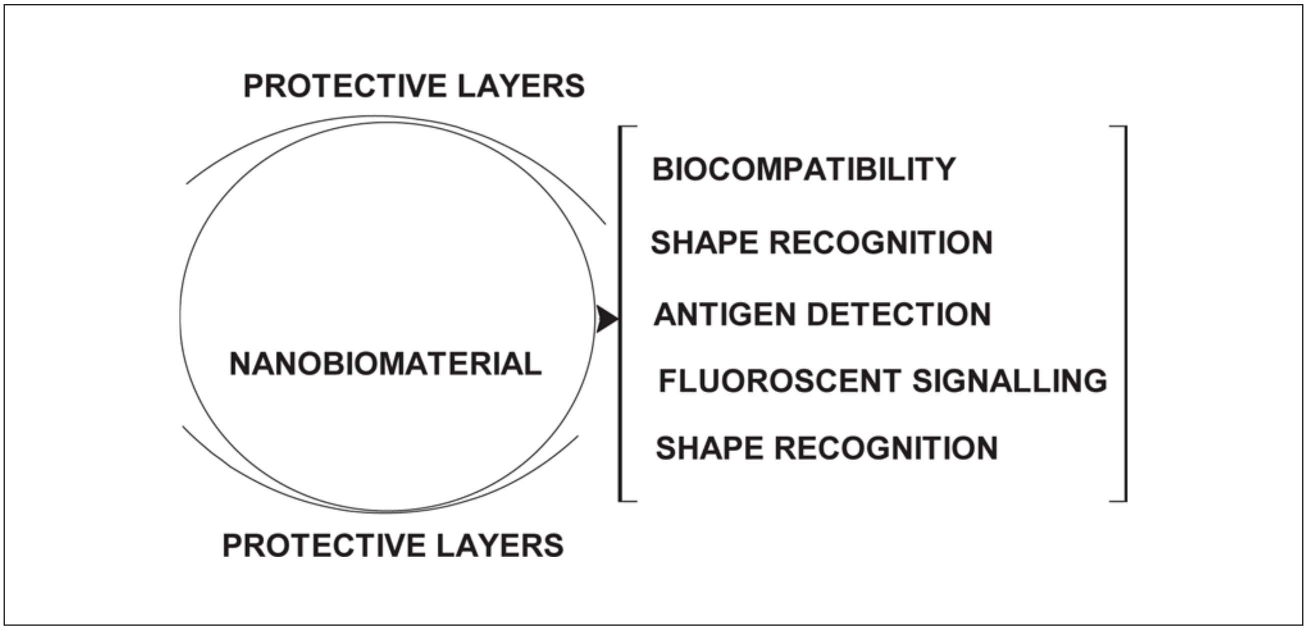

Figure 2: Specific configuration of nanobiomaterial when applied to biological or medical applications.

innovative nanomedical devices employed for drug discovery and delivery, discovery of biomarkers and molecular diagnostics.[4] Preparation methods-properties of nanoscale systems including liposomes, micelles, emulsions, nanoparticulates, and dendrimer nanocomposites, and clinical indications are known for imaging *in vivo*, sustained and targeted delivery of drugs, genes, and proteins. The recent development in drug delivery system has opened up new potential & possibility for production of pharmaceutical drugs, oral peptides, gene therapy and nanocosmeceuticals.[5] Nanobiotechnology exploits nanotechnology and biotechnology to analyze and create nanobiosystems to meet a wide variety of challenges and develops a wide range of applications. Biomaterials like DNA and proteins combined with electronic systems consequently results in the formation of new devices, sensors, and systems. Examples include (i) a carbon nanotube with single-stranded DNA wrapping around it. (ii) a graphene sheet with duplex DNA molecules. (iii) a nanoparticle attachment with antibodies, (iv) a nanorod after enzyme immobilization and (v) Streptavidin (protein) attachment to nanoparticles along with biotin (protein).[6] Several technologies, including nanoparticles and nanodevices such as nanobiosensors, nanobiochips, nanosubstances were used to improve drug discovery and development.[7]

Gold nano rod-DARPP-32siRNA complexes (nanoplexes) have been reported suitable for brain-specific delivery of appropriate siRNA for therapy of drug addiction and other brain diseases.[8] Boron nitride nanotubes are structural analogues of carbon nanotubes in nature: alternating B and N atoms entirely substitute for C atoms in a graphitic like sheet with almost no change in atomic spacing. By virtue of their magnetic properties, boron nitride nanotubes could be exploited for magnetic, physically guided, drug targeting. The interactions between boron nitride nanotubes and living cells were reported and the piezoelectric properties of boron nitride nanotubes make them attractive candidates as bionanotransducers for cell sensing and stimulation, a use which still has to be exploited.[9]

DENDRIWORMS

Dendriworms (magnetic nanoworm + dendrimer) are synthetic polymers that can be used to carry a large range of molecules such as siRNA (made up of magnetic nanoparticles as well as a fluorescent nanoparticle) which allow the nanoworm to be traced as to where it is. Tremendous progress has been achieved in the recent years in our understanding of the ability of small interfering RNAs to silence gene expression in mammalian cells. This has provided us with a revolutionary new tool to modulate the expression of disease-causing genes. Short interfering RNAs (siRNAs) have emerged as a potent new class of therapeutics, which regulate gene expression through sequence-specific inhibition of mRNA translation. Lipid-, polymer-, and nanoparticle-based siRNA delivery vehicles have proven effective in improving the stability, bioavailability, and target specificity of siRNAs following systemic administration *in vivo*. Additionally, these methods provided a platform to modify siRNAs with a variety of contrast agents and have enabled nuclear and magnetic resonance imaging of siRNA delivery in preclinical studies. Such image-guided delivery approaches represent a crucial step in the transition of siRNA therapeutics to the clinic.[10]

Scientists reported the development of dendriworms as a modular platform for siRNA delivery *in vivo*. Researchers have demonstrated that siRNA-carrying dendriworms can be readily internalized by cells and enabled endosomal escape across a wide range of loading doses, whereas dendrimers or nanoworms alone were inefficient. In addition, dendriworms carrying siRNA against the epidermal growth factor receptor reduced protein levels of epidermal growth factor receptor in human glioblastoma cells by 70−80%, 2.5-fold more efficiently than commercial cationic lipids. Reported data established dendriworms as a multimodal platform that enabled fluorescent tracking of siRNA delivery *in vivo*, cellular entry, endosomal escape, and knockdown of target proteins.[11]

INORGANIC NANOCRYSTALS

Researchers reported the first use of inorganic fluorescent lanthanide (europium and terbium) ortho phosphate [$LnPO_4 \cdot H_2O$, Ln = Eu and Tb] nanorods as a novel fluorescent label in cell biology. These nanorods, synthesized by the microwave technique, retained their fluorescent properties after internalization into human umbilical vein endothelial cells (HUVEC), 786-O cells, or renal carcinoma cells (RCC). At concentrations up to 50 μg/ml, the use of [^{3}H]-thymidine incorporation assays, apoptosis assays (TUNEL), and trypan blue exclusion illustrated the non-toxic nature of these nanorods, a major advantage over traditional organic dyes.[12]

DESIGN OF NEW-AGE DELIVERY SYSTEMS

Scientists have shown a modified version of the bacteriophage phi29 DNA-packaging motor, when reconstituted into liposomes and inserted into planar lipid bilayers, allowed the translocation of double-stranded DNA. Moreover, this engineered and membrane-adapted phage connector is expected to have applications in microelectromechanical sensing, microreactors, gene delivery, drug loading and DNA sequencing.[13]

Aptamers

Aptamers, single stranded DNA or RNA molecules, generated by a method called SELEX (systematic evolution of ligands by exponential enrichment) have been widely used in various biomedical applications. Technology combination of nanobiotechnology with aptamers opened the way to more sophisticated applications in molecular diagnosis. Recent developments in SELEX technologies and new applications of aptamers have the ability to discriminate between two closely related targets, for example a cancerous cell and an untransformed cell of the same tissue type, makes aptamers suitable as imaging reagents for non-invasive diagnostic procedures.[14]

Implantable biomedical devices

A bilayer structure comprising a thin gold layer and a polypyrrole film has been developed as a valve and holds promise for implantable biomedical devices.[15] Implantable medical devices that comprise a substrate and a porous layer comprising close packed spherical pores disposed over the substrate have been reported and the porous layer may also comprise a therapeutic agent.[16]

Nanoprobes

Tiny nanoprobes have shown to be effective in delivering cancer drugs more directly to tumor cells - mitigating the damage to nearby healthy cells - and research has shown that the nanoprobes are getting the drugs to right cellular compartments.[17] Carbon nanotubes' unique properties including low cytotoxicity and good biocompatibility attract their use as vector system in target delivery of drugs, proteins and genes.[18]

Researchers have focused on the integration of biological molecules (DNA, antibodies, and enzymes) into micro- and nanostructures, with state-of-the-art bioelectronic read-out systems, extracting useful analytical signals with interest for various fields. In this direction, nanotechnology based biosensors are the product of this integration with great interest for several applications that aim at a significant improve of the quality and security of citizen's life.[19] Rapamune was the first product based on approved NanoCrystal Technology. Nanodrugs and nanodevices approved by the FDA, and emerging nanoproducts that may pose a challenge for current regulatory schemes both in the U.S. and internationally, have been reported.[20]

Scientists have designed a novel drug delivery vehicle by hybridizing macrophages with nanoparticles through cell surface modification. Nanoparticles immobilized on the cell surface provided numerous new sites for anticancer drug loading, hence potentially minimizing the toxic effect of anticancer drugs on the viability and hypoxia-targeting ability of the macrophage vehicles. In particular, quantum dots and 5-(aminoacetamido) fluoresceinlabeled polyamidoamine dendrimer G4.5, both of which were coated with amine-derivatized polyethylene glycol, were immobilized to the sodium periodate-treated surface of RAW264.7 macrophages through a transient Schiff base linkage. Further, a reducing agent, sodium cyanoborohydride, was applied to reduce Schiff bases to stable secondary amine linkages. The distribution of nanoparticles on the cell surface was confirmed by fluorescence imaging, and it was found to be dependent on the stability of the linkages coupling nanoparticles to the cell surface.[21]

Protein therapy

Protein therapy, which delivers proteins into the cell to replace the dysfunctional protein, is considered the most direct and safe approach for treating disease and has been looked into as a possible alternative to gene therapy. Scientists have demonstrated a general, effective; low-toxicity intracellular protein delivery system based on single-protein nanocapsules, and opened a new direction not only for protein therapy but also for cellular imaging, tumor tracking, cosmetics and many other applications.[22] RNAi screen of the protein kinome identified checkpoint kinase 1 (CHK1) as a therapeutic target in neuroblastoma.[23]

Stabilizing proteins at high concentration is of broad interest in drug delivery, for treatment of cancer and many other diseases. Scientists created highly concentrated antibody dispersions (up to 260 mg/ml) comprising of dense equilibrium nanoclusters of protein [monoclonal antibody (mAb) 1B7, polyclonal sheep Immunoglobulin G (IgG) and bovine serum albumin (BSA)] molecules, which upon dilution *in vitro* or administration *in vivo*, remained conformationally stable and biologically active. The nanoclusters are formed by adding trehalose as a co-solute which strengthened the short-ranged attraction between protein molecules. The protein cluster diameter was reversibly tuned from 50 to 300 nm by balancing short-ranged attraction against long–ranged electrostatic repulsion of weakly charged protein at a pH near the isoelectric point (pI).[24]

Dendrimers/biodendrimers

Dendrimers are nanostructures produced from macromolecules such as polyamidoamine, polypropylene-imine and polyaryl ether; and are highly branched with an inner core. The particle size range is between 1 to 100 nm although their sizes are mostly less than 10 nm. Biodendrimers are dendrimers composed of repeating units known to be biocompatible or biodegradable *in vivo* to natural metabolites. Medical applications of dendrimers as multifunctional nanosized containers are shown in Figure 3.

Dendrimers have successfully proved themselves as useful additives in different routes of drug administration to be applied in routes with particular reference to intravenous, oral, transdermal, and ocular delivery systems.[25] Dendrimers can act as vectors, in gene therapy and as an agent for Photodynamic Therapy of tumorigenic keratinocytes.[26]

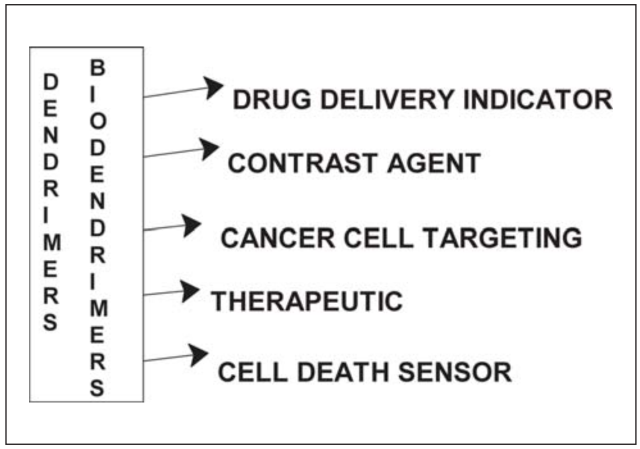

Figure 3: Some medical applications of dendrimers/biodendrimers.

NANOSTRUCTURED DRUG DELIVERY SYSTEMS

Bionanofabrication

2D crystallization of proteins, especially engineered proteins, is emerging as a powerful tool for bottom-up approaches to the nanofabrication of functional structures. Some aspects of 2D protein crystallization, including key approaches to growing 2D protein crystals and their potential applications ranging from biosensors, diagnostic kits, vaccine applications, and templates for mineral formation have been reported.[27]

Nanostructured lipid carriers (NLC) that can deliver active pharmaceutical ingredients across the skin have emerged as novel systems composed of physiological lipid materials suitable for topical, dermal and transdermal administration. The design characteristics, production and composition of semi-solid formulations containing NLC as API carriers (for example hydrogels) have been reported.[28] Results of characterization studies strongly supported the potential application of these drugs-loaded NLC as prolonged delivery systems for lipophilic drugs by several administration routes, in particular for intravenous administration.[29]

Fullerenes for Medical Diagnostics

Scientists have created new materials from Fullerenes by filling them with atoms of various metals. An important example is a fullerene that encases a sensitive contrast agent (gadolinium) for MRI applications, including as a diagnostic and therapeutic agent for brain tumors. Researchers have co-invented a hands-off process for filling fullerenes with radioactive material and these finding could be utilized in medical applications, such as MRIs and diagnostic and therapeutic agents for brain tumors.[30]

NANOBIOTECHNOLOGY AND TISSUE ENGINEERING

Scientists discussed the current applications of nanoscale materials to bladder tissue engineering.[31] Researchers explored the bottom-up and top-down nanofabrication technologies and their use in various drug delivery and tissue engineering applications.[32]

NANOBODIES

The structural properties of Nanobodies and their possible therapeutic applications were discussed and data from phase I clinical trials of the novel 'first-in-class' anti-thrombotic agent ALX-0081 (Ablynx NV) were reported.[33] Results suggested that the generation of Nanobody-displaying immune phage libraries and subsequent *in vivo* biopanning in appropriate animal models is a promising approach for the identification of novel vascular targeting agents.[34]

NANOBOTS/NANOROBOTS

Nanorobots could be employed for the diagnostics, targeted drug dispensation, elimination of xenogenous particles from the body, and repair of cells and tissues, e.g. the skin and teeth.[35] Nanorobots could also be programmed to perform delicate surgeries or remove obstructions in the circulatory system.[36]

BIOMARKERS

Biomarkers are molecules that can be measured in blood, other body fluids, and tissues to assess the presence or state of a disease. They have the potential to help us detect cancer earlier, determine a tumor's aggressiveness, or predict a patient's response to a particular treatment. Biomarkers play a role in use of pharmacogenetics, pharmacogenomics and pharmacoproteomics for development of personalized medicine. Label-free nanosensors can detect disease markers to provide point-of-care diagnosis that is low-cost, rapid, specific and sensitive. Scientists showed specific and quantitative detection of two model cancer antigens from a 10 µl sample of whole blood in less than 20 min. They used nanowire sensors to detect and measure concentrations of two specific biomarkers: one for prostate cancer and the other for breast cancer. This novel device acted as a filter, catching the biomarkers, antigens specific to prostate and breast cancer on a chip while washing away the rest of the blood.[37] The emerging trends in the development of biomarkers for early detection and precise evaluation of cancer disease were reported.[38]

This bio-nano-info convergence holds great promise for molecular diagnosis and individualized therapy of cancer and other human diseases. Scientists have demonstrated the feasibility of multiplex detection using the surface-enhanced Raman scattering-based molecular sentinel (MS) technology in a homogeneous solution. Two MS nanoprobes tagged with different Raman labels were used to detect the presence of the *erbB-2* and *ki-67* breast cancer biomarkers. The multiplexing capability of the MS technique was demonstrated by mixing the two MS nanoprobes and tested in the presence of single or multiple DNA targets.[39]

NANOBIOTECHNOLOGY IN CANCER

Application of nanotechnology/nanobiotechnology in biomedicine may contribute to significant advances in imaging diagnosis and treatment of cancer. Quantum dots, gold nanoparticles, magnetic nanoparticles, carbon nanotubes, gold nanowires and many other materials have been developed over the years, alongside the discovery of a wide range of biomarkers to lower the detection limit of cancer biomarkers. Current developments in cancer detection methods with an emphasis on nanotechnology were reported.[40] Some of the recent development in nanotechnologies and their applications in diagnosing and developing cancer therapies have been reported.[41]

Scientists reported a cooperative nanosystem consisting of two discrete nanomaterials that work in concert within the bloodstream to locate, adhere to and kill cancerous tumors. While one type of nanoparticle improved detection of the tumor, the other was designed to kill the tumor. The first component consists of gold nanorod "activators" that populate the porous tumor vessels and act as photothermal antennas to specify tumor heating via remote near-infrared laser irradiation. In addition, it was found that local tumor heating accelerated the recruitment of the second component: a targeted nanoparticle consisting of either magnetic nanoworms (NW) or doxorubicin-loaded liposomes (LP). Nine-amino acid peptide LyP-1 (Cys-Gly-Asn-Lys-Arg-Thr-Arg-Gly-Cys) was employed as the targeting species that binds to the stress-related protein, p32, which was up regulated on the surface of tumor-associated cells upon thermal treatment. Mice containing xenografted MDA-MB-435 tumors that were treated with the combined gold nanorod /LyP-1- doxorubicin-loaded liposomes therapeutic system displayed significant reductions in tumor volume compared with individual nanoparticles or untargeted cooperative system.[42]

A new nanoparticle formulation can be magnetically guided to deliver and silence genes in cells and tumors in mice. This formulation, termed LipoMag, consisted of an oleic acid-coated magnetic nanocrystal core and a cationic lipid shell. When compared with the commercially available PolyMag formulation, LipoMag displayed more efficient gene silencing in 9 of 13 cell lines, and better anti-tumour effects when systemically administered to mice bearing gastric tumours. By delivering an optimized sequence of a silencing RNA that targets the epidermal growth factor receptor of tumor vessels, the intended therapeutic benefit was achieved with no evident adverse immune reaction or untoward side effects.[43]

NANOMEDICINE

Nanomedicine has triggered the wind of revolution in medicine and important areas that nanomedicine covers include targeted drug delivery in cancer treatment, biotechnology, disease diagnosis to nanostructure implants.[44]

A variety of recent research combines Heparin (HP) and nanomaterials for a myriad of applications. HP has been conjugated to the surface of the nanoparticles, such as magnetic and metallic nanoparticles, or biodegradable and nondegradable synthetic polymers. HP has also been incorporated into the nanoparticles. The different possibilities of HP-based nanoparticle composites and their medicinal or biological applications were reported.[45]

It was qualitatively demonstrated that enhanced fluorescence emission signals occurred from clustered QDs and deduced that the band 3 membrane proteins in erythrocytes were clustered.[46]

Nanonephrology

Nanonephrology is a branch of nanomedicine and nanotechnology that deals with (i) the study of kidney protein structures at the atomic level (ii) nano-imaging approaches to study cellular processes in kidney cells and (iii) nanomedical treatments that utilize nanoparticles and to treat various kidney diseases. Various devices based on nanotechnology are used for the studying the different kidney processes and detecting disorders. Nanotechnological filters have the potential to provide immediate relief for dialysis patients.[47] Scientists have demonstrated a new artificial renal chip by integrating a high efficient biocompatible polymeric nanofibers membrane with the polydimethylsiloxane(PDMS) based micro-fluidic platform via the optimization of PDMS micro fluidic channel network and the multiple packaging of nanofibers membrane.[48]

Nanobiopharmaceutics

Nanobiopharmaceutics aims at the development of innovative multidisciplinary approaches for the design, synthesis and evaluation of molecular, nano- and micro-scale functionalities for targeted delivery of therapeutic peptides and proteins (biopharmaceutics). Nanoscale biomaterials are categorized by metal, non-metal, carbon, polymer, lipid, virus and miscellaneous nanostructures as nanobiopharmaceutical carrier systems and their medical/biological applications as well as toxicological issues in the field of biomedical nanotechnology were reported.[49] The basis of technologic application in biopharmaceutics is Nanoscale Drug Carrying System and the various units of the system consist of liposome system which varies between 30 nm to several micrometers in diameter with their characteristics governed by their size, surface composition and charge. Surface modified liposomes carrying doxorubicin and antisense oligonucleotide system have successfully targeted multidrug resistance associated protein, messenger RNA and bcl2 RNA. System after reaching the cell, delivered the doxorubicin and the antisense oligonucleotides successfully and inhibited the synthesis of MRP1 and bc12 RNA and provoked the apoptosis of carcinomatous cell by arousing the caspase (cysteine-aspartic proteases) dependent pathway.[50]

A "nanoviricide™" is a flexible nano-scale material approximately a few billionths of a meter in size, which is chemically programmed to specifically target and attack a particular type of virus like a guided missile. NV-INF-1, the selected candidate drug substance of the FluCide program, has been shown to be highly effective in controlling influenza viral infection in lethal infection mouse model. NanoViricides nanotechnology possesses potent antiviral efficacy by targeting the mechanisms by which viruses attach or bind to cells.[51]

Nanopharmacology

Nanopharmacology is the use of nanotechnology for (i) discovery of new pharmacological molecular entities; (ii) selection of pharmaceuticals for specific individuals to maximize effectiveness and minimize side effects; and (iii) delivery of pharmaceuticals to targeted locations or tissues within the body . The potential applications of biochips, nanosensors, bioreactors, neural stem cells, immune nanoparticles, biodegradable polymers, and convection-enhanced drug delivery in the diagnostics and treatment of diseases were reported. Numerous novel medicinal forms were reported, including polymeric nanoparticles, nanotubes, micelles, liposomes, dendrimers, fullerenes, and hydrogels. In particular, highly stable glycosphingolipid nanotubes and nanoliposomes were proposed as drug delivery systems. For this purpose, the model of stimulation of skin vasomotor reactions by nitroglycerin application was developed. The effect of nitroglycerin was found to increase 1.5 times when used in the form of dispersion with nanotubes as carriers and almost 2.5 times in the case of dispersion with nanoliposomes as carriers. Nanotechnologically manufactured biologically active substances Apiton-25 (containing apis products) and Microhydrin (containing SiO_2 nanoparticles, silicon hydrogen bonds Si-H, and free negative charges for free radical neutralization) results have indicated that both Apiton-25 and Microhydrin upon peroral administration enhanced the cyclic trial performance during prolonged submaximal exercise in endurance-trained cyclists.[52]

Scientists have developed a combination drug that promises a safer, more precise way for medics and fellow soldiers in battle to give a fallen soldier both morphine and a drug that limits morphine's dangerous side effects. They have used nanotechnology to devise ultra-small polymer particles capable of carrying the drugs into the body. The development of the combination drug makes possible a precise feedback system that can safely regulate release of the drugs aboard the nanoparticles.[53]

NANOBIOTECHNOLOGY AND PERSONALIZED MEDICINE

Nanobiotechnology will facilitate the integration of diagnostics with therapeutics and facilitate the development of personalized medicine.[54] Nanobiotechnology is being used to refine discovery of biomarkers, molecular diagnostics, drug discovery and drug delivery, which are important basic components of personalized medicine.[55] Personalized management of cancer, facilitated by nanobiotechnology, is expected to enable early detection of cancer, more effective and less toxic treatment increasing the chances of cure.[56]

Nanobiomaterials/Nanobioanalysis/ Nanobiochips

Novel opportunities and challenges offered by nanobiomaterials in tissue engineering have been reported.[57] The current state of development of nanodiagnostic technologies including nanobiochips and nanobiosensors has been reported.[58] Neuronanobiotechnology based delivery system are developing rapidly and one example of current nanobiotechnological research involved nanospheres coated with fluorescent polymers.[59] An assay based on gold nanoparticles could detect recurrences of prostate cancer sooner than is possible with existing techniques.[60] Carbon nanotubes have been used to probe the properties of bilayer systems resembling living cell membranes.[61]

A multifunctional one-dimensional nanostructure incorporating both CdSe quantum dots (QDs) and Fe_3O_4 nanoparticles (NPs) within a SiO_2-nanotube matrix was successfully synthesized based on the self-assembly of preformed functional NPs, allowing for control over the size and amount of NPs contained within the composite nanostructures. This specific nanostructure is distinctive because both the favorable photoluminescent and magnetic properties of QD and NP building blocks were incorporated and retained within the final silica-based composite, thus rendering it susceptible to both magnetic guidance and optical tracking. Moreover, the resulting hydrophilic nanocomposites were found to easily enter into the interiors of HeLa cells without damage, thereby highlighting their capability not only as fluorescent probes but also as possible drug-delivery vehicles of interest in nanobiotechnology.[62]

A large variety of nanobioanalysis methods have been reported.[63] Scientists have described the construction and use of two major classes of nano-bio-chip designs that serve as cellular and chemical processing units. These nanobiochips possess capabilities for measuring such diverse analyte classes as cells, proteins, DNA and small molecules in the same compact device. Further, applications such as disease diagnosis and prognosis for areas including cancer, heart disease and HIV were reported.[64] One-dimensional nanostructures such as nanowires are ideal for diagnosis as they can be integrated into microfluidic chips that provide a complete sensor system.[65]

DNA nanomachines are synthetic assemblies that switch between defined molecular conformations upon stimulation by external triggers. Researchers have reported the construction of a DNA nanomachine called the I-switch, which is triggered by protons and functions as a pH sensor based on fluorescence resonance energy transfer inside living cells. Moreover, this was found an efficient reporter of pH from pH 5.5 to 6.8, with a high dynamic range between pH 5.8 and 7, thereby, illustrating the potential of DNA scaffolds responsive to more complex triggers in sensing, diagnostics and targeted therapies in living systems.[66]

INTEGRATION OF RECOMBINANT TECHNOLOGY AND NANOSCIENCE

Recombinant technology is the most important prerequisite for the effective engineering of nano-structured deoxyribonucleic acid and protein based materials in nanoscience. This technology allowed the manipulation of the properties of molecules, including physico-chemical properties of proteins that control electron transport and photochemical processes in the development of molecular electronic devices and device fabrication. Recombinant molecules, such as recombinant ovalbumin and recombinant ovalbumin mutants have provided a powerful means for the study of their physico-chemical and structural characteristics, and thereby for their use in nanoscience. The researchers have provided an overview of the integration of recombinant technology and nanoscience through reported studies in areas, including food, environment, medicine, physics and chemistry.[67]

CONCLUSIONS AND PERSPECTIVES

Nanobiotechnology is an emerging field that seeks new solutions to pressing health and environmental problems by combining physical sciences and engineering with life sciences and medicine. This exciting frontier of discovery is generating new therapies, devices, diagnostic tools, and a better understanding of the relationship between cells and disease. Nanobiotechnology also deals with the investigation and utilization of the newly conceived nanomaterials, as well as the construction of functionalized nanobiosystems. Nanopharmaceuticals offer the ability to detect diseases at much earlier stages. Drug delivery is the most advanced tool of nanobiotechnology. Nanomedicine combines nanotechnology and medicine, and nanotherapeutics is the use of nanomedicine in therapy. The ultimate goal of nanotherapeutic is comprehensive monitoring repair and improvement of all human biologic system. Nanobiotechnology should provide many of the tools necessary to enable the components that will one day be inserted into commercialized products.

Genomics, proteomics, and metabolomics combined with the power of nanobiotechnology have the potential to understand the disease in a way that was previously not possible and it is expected that the disease will be targeted more effectively and precisely. Experts support a comprehensive technology assessment of nanobiotechnologies along with a full-blown risk/benefit analysis before their adoption. New biofriendly imaging probes based on functionalized inorganic nanocrystals are being developed and are available to facilitate state-of-the-art bioimaging studies. The research on the combination of chemotherapeutic agents with gene therapy should be further strengthened to overcome the limitations of conventional cancer treatment. In this context, a strongly synergistic antiproliferative effect was observed in colon cancer cells when *E* gene expression was combined with the activity of the 5-Fluorouracil–loaded biodegradable poly(ε-caprolactone) nanoparticles, thereby indicating the potential therapeutic value of the combined therapy.[68] It is hoped that nanobiotechnology will extend the limits of current molecular diagnostics and enable point of care diagnostics, integration of diagnostics with therapeutics, and development of personalized medicine. Three-dimensional nanobiostructure-based self-contained devices consisting of a glucose/oxygen sensor, a biofuel cell and a wireless signal transmitter to demonstrate wireless monitoring of glucose and oxygen in biological fluids, wounds, and cell cultures is under development.[69] As yet there are no directives to regulate nanobiotechnology by various regulating bodies but as products are ready to enter market, these are expected to be in place. The largest expansion is expected in coming years.[70] Nanobiotechnology is likely to trigger advances in the early detection of a variety of diseases and improvements in biological implants. Experts are of the opinion that efforts should be made to educate, and increase awareness about nanobiotechnology through a transparent public dialogue.

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