The role of nanobiotechnology in drug discovery

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The application of nanotechnology in life sciences, nanobiotechnology, is already having an impact on diagnostics and drug delivery. Now, researchers are starting to use nanotechnology in the field of drug discovery. This review explains how several technologies, including nanoparticles and nanodevices such as nanobiosensors and nanobiochips, are used to improve drug discovery and development. Nanoscale assays can contribute significantly to cost-saving in screening campaigns. In addition, some nanosubstances (such as fullerenes) could be potential drugs for the future. Although there might be some safety concerns with respect to the *in vivo* use of nanoparticles, studies are in place to determine the nature and extent of adverse events. Future prospects for the application of nanotechnology in healthcare and for the development of personalized medicine appear to be excellent.

The current drug discovery paradigm constantly needs to progress, increasing efficiency and reducing time to market. The post-genomic era has uncovered many potentially important targets. However, to exploit their value in full, the efficiency of screening and validation processes must be improved. This review explains how nanotechnology can play a role in improving the drug discovery process.

Nanotechnology is the creation and utilization of materials, devices and systems through the control of matter on the nanometer scale. Given the inherent nanoscale functions of the biological components of living cells, it was inevitable that nanotechnology would be applied to the life sciences. Such applications give rise to the term nanobiotechnology [1]. Analyses of signaling pathways by nanobiotechnology techniques might provide new insights into disease processes, thus identifying more efficient biomarkers and understanding the mechanisms of action of drugs. This will help in designing new approaches to drug discovery.

Technical achievements in nanotechnology are being applied to improve drug discovery, drug delivery and pharmaceutical manufacturing. It is a common misconception that most of the applications of nanobiotechnology in drug discovery involve the use of nanoparticles. There are several fundamental nanotechnologies and nanodevices that can be used in drug discovery and these have been described in more detail elsewhere [1]. Applications of various nanotechnologies are described, according to the stages of drug discovery. Some of these technologies are nanoscale miniaturizations of microfluidic technologies such as those used in proteomics.

Nanotechnological applications in target identification and validation

Proteomic applications

Proteomics is playing an important role in the target identification and validation phases of the drug discovery process. Most current protocols, including protein purification and/or display and automated

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identification schemes, yield unacceptably low recoveries, thus, limiting the overall process with respect to sensitivity, speed and the requirement of large amounts of starting material. Less abundant proteins and proteins that can only be isolated from source materials that are limited in quantity (e.g. biopsies, body fluids) can be subjected to nanoscale protein analysis, nanocapture of the specific proteins and/or complexes and the optimization of all subsequent sample-handling steps, leading to the molecular-mass determination of the peptide fragments. Some nanotechnologies are refining the application of proteomics for drug discovery and examples are described briefly in this review.

Nanodevices

Use of nanotube electronic biosensors in proteomics

Single-walled carbon nanotubes have been used as a platform for investigating surface–protein and protein–protein binding, as well as to develop highly specific electronic biomolecule detectors [2]. Non-specific binding on nanotubes, a phenomenon found with a wide range of proteins, is overcome by the immobilization of polyethylene-oxide chains. A general method is followed that enables the selective recognition and binding of target proteins, conjugating their specific receptors to polyethylene-oxide functionalized nanotubes. These arrays are attractive because no labeling is required and the entire assay can be done in the solution phase. This scheme, combined with the sensitivity of nanotube electronic devices, provides highly specific electronic sensors for detecting clinically important biomolecules (such as antibodies associated with human autoimmune diseases).

Nanotechnologies

High-field asymmetric waveform ion mobility spectrometry

An ion mobility technology, high-field asymmetric waveform ion mobility spectrometry (FAIMS), has been introduced as an online ion-selection method that is compatible with electrospray ionization (ESI). FAIMS uses ion separation to improve the detection limits of peptide ions, when it is used in conjunction with electrospray and nanoelectrospray mass spectrometry (MS). This facilitates the identification of low-abundance peptide ions, often present in part per million (ppm) levels as complex proteolytic digests, and expands the sensitivity and selectivity of nano liquid chromatography mass spectrometry (LC–MS) analyses in global and targeted proteomics approaches. This functionality will probably play an important role in drug discovery and in biomarker programs for monitoring disease progression and drug efficacy [3].

Investigating biomolecular interactions with atomic force microscopy

Atomic force microscopy (AFM) is a well-established technique for imaging single biomolecules under physiological conditions. The exceptionally high spatial resolution and signal-to-noise ratio obtained with AFM enables the substructure of individual molecules to be observed. Used as a sensor, the AFM tip can probe the charges of biological surfaces immersed in a buffer solution. So far, such approaches have successfully characterized protein interactions but in the future they could be applied to the imaging and the detecting of multiple parameters on a single molecule simultaneously.

An approach called topography and recognition imaging (TREC) uses a ligand (such as an antibody, a small organic molecule or a nucleotide), bound to a carefully designed AFM tip-sensor, to estimate affinity and structural data through a series of unbinding experiments [4]. If a ligand is attached to the end of an AFM probe, one can simulate various physiological conditions and look at the strength of the interaction between the ligand and receptor in a wide range of circumstances. By functionalizing the tip, it can be used to probe biological systems and to identify particular chemical entities on the surface of a biological sample. This opens the door to more-effective uses of AFM in drug discovery.

AFM has been used to study the molecular-scale processes underlying the formation of the insoluble plaques associated with Alzheimer's disease (AD). As one of a class of neurological diseases caused by changes in the physical state of a protein (conformational diseases), it is particularly well-suited for study with AFM. Extensive data suggest that the conversion of the Aβ peptide from its soluble to its insoluble form is a key factor in the pathogenesis of AD. In recent years, AFM has provided useful insights into the physicochemical processes involved in Aβ plaque formation. AFM was crucial for identifying the nanostructures, now widely recognized as essential, in amyloidogenesis in AD. The technique has revealed other forms of aggregation, observed at earlier disease-stages, which evolve to associate into mature fibrils. Now, AFM can be used to explore factors that either inhibit or promote fibrillogenesis. Using AFM enables two monoclonal antibodies (being studied as potential treatments for AD) to be compared, selecting the one that is better at inhibiting the formation of these protofibrils. The first antibody, m266.2, binds to the central portion of the Aβ peptide and completely inhibits the formation of protofibrils, whereas the other antibody, m3D6, retards (but does not totally stop) protofibril growth [5]. These results indicate that AFM can be used reliably to study the effect of different molecules on Aβ peptide aggregation and that it can provide additional information, such as the role of epitope specificity for antibodies as potential inhibitors of fibril formation.

Examining molecular reactions and interactions using cantilevers

Cantilevers, developed by Concentris GmbH (Basel, Switzerland), transform a chemical reaction into a mechanical motion on the nanometer scale. The measurements

of a cantilever are: length 500 μm; width 100 μm; thickness 25–500 μm; and deflection 10 nm. This motion can be measured directly by deflecting a beam of light from the surface of the cantilever. Concentris use an array of parallel vertical-cavity surface-emitting lasers (VCSELs) as a stable, robust and proven light-source. A state-of-the-art position-sensitive detector is employed as the detection device.

The static mode of the cantilever is used to obtain information regarding the presence of certain target molecules in the sample under investigation. The surface stress caused by the adsorption of these molecules results in minute deflections of the cantilever and these deflections correlate directly to the concentration of the target substance. The dynamic mode quantitatively analyzes mass-loads in the sub-picogram region and, as molecules are adsorbed, measures minimal shifts in the resonance frequency of the oscillating cantilever, associating this with reference data for the target substance. Both modes can be operated simultaneously and the controlled deposition of functional layers is the key to converting nanomechanical cantilevers into chemical or biochemical sensors. Inkjet printing is a rapid method used to coat cantilever arrays efficiently with various sensor layers [6]. Applications relevant to drug discovery include label-free biochemical assays and investigating biomolecular interactions as well as multiplexed assays. By attaching specific antibodies to cantilevers, the simultaneous imaging of target antigens and identification of antigen–antibody interactions have been demonstrated [7].

Nanotechnological applications in lead identification

Nanodevices

Biosensors

Biosensors are currently used in the areas of target identification, validation, assay development, lead optimization and absorption, distribution, metabolism, excretion and toxicity (ADME-Tox). They are best-suited to applications using soluble molecules and overcome many of the limitations that arise with cell-based assays. They are particularly useful in the study of receptors because they do not require the receptor to be removed from the lipid membrane of the cell, which can be necessary with other assay methods. A primary application of the current biosensor technologies is the optimization of limited-scope drug libraries against specific targets.

A novel nanobiosensor (based on magnetic nanoparticles) has been developed for rapid screens of telomerase activity in biological samples [8]. The technique utilizes nanoparticles that, upon annealing to telomerase-synthesized TTAGGG repeats, switch their magnetic state (a phenomenon readily detectable by magnetic readers). A high-throughput adaptation of this technique, utilizing magnetic resonance imaging for the purposes of detection, allows processing of hundreds of samples within tens of minutes at ultrahigh sensitivities. Together, these studies establish and validate a novel, powerful tool for rapidly sensing telomerase activity and they provide the rationale for developing analogous magnetic nanoparticles for *in vivo* sensing. Because elevated telomerase levels are found in many malignancies this technique provides access to an attractive target for diagnosis, as well as therapeutic intervention.

Nanowire devices

The development of miniaturized devices that enable rapid and direct analysis of the specific binding of small molecules to proteins could be significant in the screening and discovery of new drugs. Sensitive, label-free, direct electrical detection of small-molecule inhibitors of ATP binding to Abl has been reported, using silicon nanowire field-effect transistor devices [9]. These researchers covalently linked Abl, a protein tyrosine kinase with constitutive activity that is responsible for chronic myelogenous leukemia, to the surfaces of silicon nanowires within microfluidic channels to create active electrical devices. They monitored the nanowire conductance to assess the concentration-dependent binding of ATP and its inhibition by the competitive small-molecule antagonist Gleevec®. In addition, concentration-dependent inhibition of ATP binding was examined for four additional small molecules, including reported and previously unreported inhibitors. These studies demonstrated that the silicon nanowire devices can readily and rapidly distinguish among the affinities of distinct, small-molecule inhibitors, therefore, serving as a technology platform for drug discovery.

Nanotechnologies

Assays based on endocytosis at the nanoscale

Nanobiotechnology creates a better understanding of cell biology because the molecules in the cell are organized in nanometer-scale dimensions and they function as nanomachines. It provides an insight into processes involved in endocytosis. Clathrin-mediated endocytosis (CME) plays a fundamental role in many cellular activities including receptor downregulation, nutrient uptake and the maintenance of signal transmission across nerve-cell junctions. Disturbances in CME are implicated in cancer and neurodegenerative diseases. Live cell imaging, along with a novel fluorescence assay, has been used to visualize the formation of clathrin-coated vesicles at single clathrin-coated pits (CCP) with a time resolution of seconds [10]. This reveals how proteins linked to actin, part of the molecular motor, are transported to sites of the coated pit. Disrupting actin polymerization with the toxin latrunculin B (a toxin found in Red Sea sponge) drastically reduces the efficiency of membrane scission and affects many aspects of CCP dynamics. The novel assay used in this study can be applied for drug screening.

Electron microscopy has uncovered new structures (~40 nm in size) that are involved in endocytosis and can be hijacked by viruses to gain entry to cells [11]. An understanding of this process could provide a route to deliver drugs into cells or could assist in the development of new antiviral drugs.

TABLE 1

Selected companies with nanoliter and nanobiochip technologies relevant to pharmaceutical applications

Company and/or laboratory	Technology	Applications
Advalytix (Brunnthal, Germany)	Microagitation chips: nanopumps using surface-acoustic waves can move nanoliter droplets to control mixing and subsequent chemical reactions	Make DNA and protein microarrays a reliable tool for pharmaceutical research and a high throughput platform
BioForce Nanosciences (Ames, IA, USA)	NanoArray™: 400 nanoarray spots can be placed in the same area as a traditional microarray and as many as 1500 different samples can be queried	Drug discovery
	Protein nanoarrays contain up to 25,000,000 spots per cm^2	To detect protein–protein interactions
Caliper Life Sciences (Hopkinton, MA, USA)	LabChip and Sciclone® liquid-handler platform: handles volumes in the nanoliter range	Molecular diagnostics
Drug Discovery CombiMatrix Corporation (Mukilteo WA, USA)	NanoArrays™: microelectrodes and electrochemical synthesis	Life-sciences research and drug discovery
Labcyte (Sunnyvale, CA, USA)	Use of focused acoustic energy to eject nanoliter transfer-volumes of liquid	High-density microarrays. Pharmaceuticals particle manufacture
Nanogen (San Diego, CA, USA)	NanoChip system: integrates advanced microelectronics and molecular biology	Genomics, genetic testing and drug discovery
Nanolytics (Raleigh, NC, USA)	Biochip containing 10,000 nanodroplet wells and a computer-controlled microactuator to move and position the nanodroplets independently	Assay density is increased 50–200 fold compared with traditional plates and all reagents can be decreased by 1000 fold in drug discovery
Nanosyn (Menlo Park, CA, USA)	Accelerated Nanoscale Synthesis Technology (ANST™) and nanoscale libraries	Automated high-throughput, drug discovery and cost reduction
SuNyx (Cologne, Germany)	Nanostructured surfaces for nanofluidic bioanalytics	Analytical biochips. Pharmaceutical manufacture

Surface plasmon resonance

Optical biosensors capable of exploiting surface plasmon resonance (SPR), waveguides and resonant mirrors have been used a lot over the past decade to analyze biomolecular interactions. These sensors determine the affinity and kinetics of a wide variety of molecular interactions in real time, without the need for a molecular tag or label [12]. Conventional SPR is applied in specialized biosensing instruments that use expensive sensor chips of limited reusable capacity and require complex chemistry for ligand or protein immobilization. SPR has also been successfully applied with colloidal gold particles in a buffered solution [13]. This application offers many advantages over conventional SPR because the support is cheap, easily synthesized and can be coated with various proteins or protein–ligand complexes, by charge adsorption. Using colloidal gold, the SPR phenomenon can be monitored in any UV–visible spectrophotometer. For high-throughput applications, the technology has been adapted in an automated clinical-chemistry analyzer. Among the label-free systems currently available, the use of metal nanocolloids offers enhanced throughput and flexibility for real-time biomolecular-recognition monitoring, at a reasonable cost [14]. A sensitive technique is being developed for the optical detection of gold nanoparticle-labeled molecules on protein microarrays by applying the SPR and specific molecular binding using rolling-circle amplification [15].

Nanofluidics, nanoarrays and nanobiochips

Nanofluidics implies extreme reduction in quantity of fluid analyte in a microchip compared with standard methodologies. The use of the word 'nano' in nanoliter is in a different dimension to that in nanoparticle, which is in the nanometer scale. Chemical compounds within individual nanoliter droplets of glycerol have been microarrayed on to glass slides at 400 spots/cm^2 [16]. This technique enables the kinetic profiling of protease mixtures, protease–substrate interactions and high-throughput screening reactions. From one printing run (that consumes <1 nanomole of each compound) large combinatorial libraries can be subjected to numerous separation-free homogeneous assays at volumes that are a small fraction of those used in current high-throughput methods.

Nanoarrays are the next stage in the evolution of the miniaturization of microarrays. Whereas microarrays are prepared by robotic spotting or optical lithography, limiting the smallest size to several microns, nanoarrays require further developments in lithography strategies such as electron-beam lithography, dip-pen nanolithography, scanning-probe lithography, finely focused ion-beam lithography and nanoimprint lithography. Nanoarrays can measure interactions between individual molecules down to a resolution of just one nanometer and can be used in bioaffinity tests for proteins, nucleic acids and receptor–ligand pairs. Companies with nanoliter biochip technologies, applicable to drug discovery, are shown in Table 1.

Miniature devices are being constructed to study synthetic cell membranes in an effort to speed up the discovery of new drugs for a variety of diseases, including cancer. Cell membranes contain a variety of proteins, some of which act as tiny pumps that quickly remove chemotherapy drugs

from tumor cells, making the treatment less effective. Research aims to find drugs that deactivate the pumps, making chemotherapy drugs more effective. A chip constructed for research in this area measures ~1 cm^2 and holds 1000s of cylindrical cavities, open at the top but sealed at the bottom with an inorganic, porous alumina membrane that separates the reaction chamber from a solution [17]. The pores in this membrane are <100 nm in size, so they do not allow proteins to pass readily through the membrane but they will allow smaller molecules to do so. An enzyme is placed inside, onto the inner surface of the alumina membrane and the liquid containing the molecules to be tested is placed outside each vessel so that it covers the opposite side of the membrane. When the liquid diffuses through the pores, it mixes with the enzyme triggering a reaction that produces a blue color. The ability to perform reactions inside the reactor means that it is possible to do assays that could not be done previously in such a small device. The goal is to produce 'laboratories-on-a-chip', which might contain up to a million test chambers, or 'reactors', each capable of screening an individual drug. The chips could dramatically increase the number of experiments possible with a small amount of protein. This device will facilitate the study of membrane proteins, important because many future drugs designed to treat disease probably work by controlling proteins in cell membranes.

Nanoflow liquid chromatography

The use of liquid chromatography (LC) in analytical chemistry is well established but the relatively low sensitivity associated with conventional LC makes it unsuitable for the analysis of some biological samples. Furthermore, standard LC flow rates are frequently not compatible with the use of specific detectors, such as electrospray ionization mass spectrometers. Therefore, owing to the analytical demands of biological samples, miniaturized LC techniques were developed to allow for the analysis of samples with greater sensitivity than that afforded by conventional LC. In nanoflow LC (nanoLC), chromatographic separations are performed using flow rates in the low nanoliter per minute range, which result in high analytical sensitivity due to the large concentration efficiency afforded by this type of chromatography. NanoLC, in combination with tandem mass spectrometry, was first used to analyze peptides and (as an alternative to other mass spectrometric methods) to identify gel-separated proteins. Gel-free analytical approaches, based on LC and nanoLC separations, have been developed and are allowing proteomics to be performed in a faster and more comprehensive manner than strategies based on the classical 2D gel electrophoresis approaches allow [18]. Protein identification using nanoflow liquid chromatography–tandem mass spectrometry (LC–MS–MS) provides reliable sequencing information for low femtomole-level protein digests. However, this task is more challenging for subfemtomole peptide levels.

Nanotechnological applications in lead optimization

Nanoparticles

A few articles published on nanotechnology as applied to drug discovery focus on nanoparticles [19]. No single type of nanoparticle is suitable for universal application in drug discovery. Older imaging tools, such as fluorescent dyes or polymer spheres, are either too unstable or too big to perform single-molecule tracking effectively but quantum dots (QDs) are useful for this purpose. The use of QDs for drug discovery has been explored extensively and an example of their use is imaging drug receptors in the brain.

Quantum dots for imaging drug receptors in the brain

QDs are used to track individual glycine receptors (GlyRs) and to analyze their dynamics in the neuronal membrane of living cells, for periods ranging from milliseconds to minutes [20]. The GlyRs are the main inhibitory neurotransmitter in the human spinal cord and brain stem. The entry of GlyRs into a synapse by diffusion was observed and further confirmed by electron-microscopy imaging of QD-tagged receptors.

Several QDs are commercially available. Qdot™ conjugates, from the Quantum Dot Corporation (Hayward, CA, USA), can produce photo resolutions up to eight times more detailed than the older imaging tools. The Qdot™ conjugates also proved to be almost an order of magnitude brighter than fluorescent dyes and can be observed for as long as 40 min compared with ~5 s for the fluorescent dyes. The length of the observation time is crucial for studying cellular processes, which change rapidly over a span of several minutes. Because cellular receptors are crucial targets for new drug candidates, a more detailed understanding of the behavior of these receptors can open up new treatment options.

Gold nanoparticles

Gold nanoparticles are the most commonly used nanomaterial in diagnostics; they have many other uses as well. They are also used as a connecting point to build biosensors to detect disease DNA. Instead of a fluorescent molecule, a gold nanoparticle can be attached to an antibody and other molecules, such as DNA, can be added to the nanoparticle to produce bar codes. Because many copies of the antibodies and DNA can be attached to a single nanoparticle, this approach is much more sensitive and accurate than the fluorescent-molecule tests used currently. Although they can be used for drug discovery, they need to be combined with another technology for visualization.

Gold nanoparticles have been used to demonstrate multiphoton absorption-induced luminescence (MAIL), where specific tissues or cells are fluorescently labeled using special stains that enable them to be studied. Gold nanoparticles emit light, so intense that it is easily possible to observe a single nanoparticle at laser intensities lower than those commonly used for MAIL (i.e. sub-100 fs pulses of 790 nm light) [21]. Moreover, gold nanoparticles

do not 'blink' or 'burn out', even after hours of observation. The observations suggest that metal nanoparticles are a viable alternative to fluorophores or semiconductor nanoparticles for biological labeling and imaging. Other advantages of the technique are: the gold particles can be prepared easily; they have very low toxicity; and they can be attached readily to molecules of biological interest. In addition, the laser light used to visualize the particles is at a wavelength that causes only minimal damage to most biological tissues. This technology could enable the tracking of a single drug molecule in a cell or in other biological samples.

Lipoparticles for drug discovery

The lipoparticle technology used at Integral Molecular Inc (Philadelphia, PA, USA) enables integral membrane proteins to be solubilized while retaining their intact structural conformation. Retaining the native structural conformation of membrane-bound receptors is essential during assay development for optimal lead selection and optimization. This approach was developed initially to study ligands binding to membrane proteins in HIV-1, including Env co-receptor interactions using an optical biosensor. CCR5, CXCR4 and other membrane proteins were incorporated into retrovirus particles that were purified and attached to the biosensor surface [22]. Binding of conformationally sensitive antibodies, as well as Env, to these receptors was readily detected. This approach has implications for drug discovery in which binding of small molecules to 7-transmembrane (and other membrane) receptors could be measured. Lipoparticles can be paired with a multitude of detection systems including biosensors and they will be used for identification and optimization of chemical compounds (for antibody development) and to purify and concentrate structurally intact receptors from naturally occurring cell lines.

Nanotechnologies

Fluorescence planar wave guide technology

The fluorescence planar wave guide (PWG) technology, used in ZeptoMARK™ protein microarrays from Zeptosens AG (Witterswil, Switzerland), uses a 150–300 nm film of a material with a high refractive index that is deposited on a transparent support with a lower refractive index (e.g. glass or polymer). A parallel laser light beam is coupled into the waveguiding film by a diffractive grating that is etched or embossed into the substrate. A variety of proteins can be immobilized on PWG microarrays as selective recognition elements for the investigation of specific ligand–protein interactions (e.g. antigen–antibody, protein–protein and protein–DNA interactions). Protein microarrays based on PWG allow the simultaneous, qualitative and quantitative analysis of protein interactions with high sensitivity in a parallel manner. This method enables cost-effective determination of drug-candidate efficacy in a vast number of preclinical study samples.

Nanomaterials as drug candidates

Dendrimers

Dendrimers are a novel class of 3D, nanoscale, core-shell structures that can be synthesized for a wide range of applications. Specialized chemistry techniques allow precise control over the physical and chemical properties of the dendrimers. The techniques are especially useful in drug delivery but can also be used for the development of new pharmaceuticals with novel activities. Polyvalent dendrimers interact simultaneously with multiple drug targets. They can be developed into novel, targeted cancer therapeutics. Dendrimers can be conjugated to different biofunctional moieties, such as folic acid using cDNA oligonucleotides, to produce clustered molecules targeting cancer cells that overexpress the high-affinity folate receptor [23,24].

Fullerenes

An important feature of fullerene molecules is that they have numerous points of attachment, allowing for precise grafting of active chemical groups in three-dimensional orientations. This attribute, the hallmark of rational drug design, allows positional control in matching fullerene compounds to biological targets. Together with other attributes, namely the size of the fullerene molecules, the redox potential and the relative inertness in biological systems, it is possible to tailor requisite pharmacokinetic characteristics to fullerene-based compounds and to optimize their therapeutic effect [25].

Fullerene antioxidants bind and inactivate multiple, circulating, intracellular free radicals. This binding gives them unusual power to stop free-radical injury and to halt the progression of diseases caused by excess free-radical production. Fullerenes provide an effective defense against all of the principal, damaging forms of reactive oxygen species. C60 fullerene has 30 conjugated carbon–carbon double bonds; all of them can react with a radical species. In addition, the capture of radicals by fullerenes is too fast to measure and is referred to as being 'diffusion controlled'. This means the fullerene forms a bond with a radical every time it encounters one. Numerous studies demonstrate that fullerene antioxidants work much better as therapeutics than other natural and synthetic antioxidants do, at least for CNS-degenerative diseases. In oxidative injury or disease, fullerene antioxidants enter cells and modulate free-radical levels, thereby substantially reducing or preventing permanent cell injury and cell death (mechanisms of action of fullerene are shown in Box 1).

Fullerenes have potential applications in the treatment of diseases where oxidative stress plays a role in the pathogenesis (e.g. neurodegenerative diseases). Another possible application of fullerenes is in nuclear medicine as an alternative to chelating compounds that prevent the direct binding of toxic metal ions to serum components. This could increase the therapeutic potency of radiation treatments and decrease their adverse effects because fullerenes are resistant to biochemical degradation within the body.

BOX 1

Mechanism of action of fullerenes

Fullerenes can capture multiple electrons derived from oxygen free-radicals in unoccupied orbitals.

When an attacking radical forms a bond with fullerene it creates a stable and relatively nonreactive fullerene radical.

A tris-malonic acid derivative of the fullerene C60 molecule (C3) is capable of removing the biologically important superoxide radical.

C3 localizes to mitochondria, suggesting that C3 functionally replaces manganese superoxide dismutase (SOD), acting as a biologically effective SOD mimetic [30].

BOX 2

Advantages of nanobodies relevant to developing therapeutics

- They are extremely stable and bind antigen with nanomolar affinity.
- They have high target specificity and low inherent toxicity.
- They can be humanized
- They combine the advantages of conventional antibodies with important features of small-molecule drugs.
- They can address therapeutic targets not easily recognized by conventional antibodies (e.g. active sites of enzymes).
- They can be administered by routes other than intravenous administration
- They can be produced cost effectively on a large scale.
- They have an extremely low immunogenic potential.
- They have the ability to cross the human blood–brain barrier to reach targets in the brain.

Nanobodies

Nanobodies produced by Ablynx (Ghent, Belgium) are the smallest, available, intact, antigen-binding fragments harboring the full antigen-binding capacity of the naturally occurring heavy-chain antibodies. Nanobodies have the potential to be a new generation of antibody-based therapeutics and to be used in diagnostics, for diseases such as cancer [26]. Advantages of nanobodies relevant to development of therapeutics are shown in Box 2.

Nanobiotechnology and drug discovery for personalized medicine

The term personalized medicine simply means the prescription of specific treatments and therapeutics best suited for an individual [27]. It is also referred to as individualized or individual-based therapy. Personalized medicine is based on the idea of using a patient's genotype as a factor in deciding on treatment options (but other factors are also taken into consideration). Molecular diagnostics is an important component of personalized medicine and nanobiotechnologies are already being used in molecular diagnostics [28]. Although current efforts using pharmacogenomics and pharmacogenetics include matching the existing drugs to the right patients for optimal efficacy and safety, future personalized medicines could be discovered and designed for specific groups of patients using pharmacoproteomics [29]. Nanobiotechnology promises to facilitate discovery of personalized medicines (apart from facilitating the integration of diagnostics and therapeutics).

Nanobiotechnology for combination of drug design and drug delivery

Many drugs discovered in the past could not be used in patients because a suitable method of drug delivery was lacking. Nanotechnology is also used to facilitate drug delivery. A product incorporating the NanoCrystal® technology of Elan Drug Delivery (King of Prussia, PA, USA), a solid-dose formulation of the immunosuppressant sirolimus, was approved by the FDA in 2000. Abraxane™ (Abraxis™ Oncology), containing paclitaxel as albumin-bound particles in an injectable suspension, is approved for the treatment of breast cancer after the failure of combination chemotherapy for metastatic disease or after relapse within six months of adjuvant chemotherapy. It is based on nanoparticle technology, which integrates biocompatible proteins with drugs to create the nanoparticle form of the drug (with a size ~100–200 nm) to overcome the insolubility problems encountered with paclitaxel. Now, the trend is to consider drug-delivery issues at the earlier stages of drug discovery and design. Potential applications of nanotechnology to facilitate drug delivery can be taken into consideration at the stage of drug design. A carrier nanoparticle can be designed simultaneously with the therapeutic molecule.

An example is the approach of Calando Pharmaceuticals (Duarte, CA, USA), to design short interfering RNAs (siRNAs) as anticancer agents using nanoparticle delivery. Proprietary technology uses sequence selection and construction of effective siRNA molecules that bind to (and self-assemble with) the siRNA to form uniform colloidal-sized particles ~50 nm in diameter. As they are administered by intravenous injection, larger particles cannot get out of the blood and penetrate the tumor. If the drug particles are smaller than 10 nm they are quickly excreted through the kidneys.

Conclusions

The examples given in this review cover several different nanotechnologies. Some of these are already established in research through well-known technologies such as biosensors and biochips. Nanoparticles are still used extensively for developing diagnostics and some of the assays for drug discovery.

Limitations of use of nanotechnologies for drug discovery

The nanoparticles available are not ideal for all of the requirements of drug discovery and the choice of nanoparticle will depend on the individual needs. QDs can be used for high-throughput cell-based studies with the advantage

of multiplexing (i.e. multiple leads can be tested at the same time). However, as discussed earlier in this review, there are some limitations still to be resolved for their use in the drug-discovery studies such as toxicity, size variation, agglomeration, potential multiple drug attachment to a single QD and blinking.

With a large number of nanotechnologies and nanomaterials, no generalizations can be made about safety and toxicity. *In vitro* diagnostic use does not pose any safety risks to people but there is a concern over the *in vivo* use of nanoparticles, particularly those <50 nm in size, which can enter the cells and there are still many unanswered questions about their fate in the living body. Because of the huge diversity of materials used and the wide range in sizes of nanoparticles, these effects will vary a lot. It is conceivable that particular sizes of some materials might turn out to have toxic effects and further investigations will be needed. The FDA approval is essential for clinical applications of nanotechnology and substantial regulatory problems could be encountered in the approval of nanotechnology-based products. Pharmaceuticals, biologicals and devices are all regulated differently by the FDA and it is not yet clear how emerging nanotherapeutics will be evaluated.

Future of nanotechnology-based drug discovery

An increasing use of nanobiotechnology by the pharmaceutical and biotechnology industries is anticipated. Apart from innovations based on nanoparticles, several other nanotechnologies are in development for use in life sciences. In the near future, it might be possible to accurately model the structure of an individual cell and to predict its function using computers connected to nanobiotechnology systems. Such a detailed virtual representation of how a cell functions might enable scientists to develop novel drugs with unprecedented speed and precision, without doing any experiments in living animals.

Nanotechnology will be applied at all stages of drug development, from formulations for optimal delivery to diagnostic applications in clinical trials. It will fit in with the concepts for the integration of diagnostics and therapeutics to develop personalized medicine.

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