Chapter 5
Nanopharmaceuticals

Introduction

The term "nanopharmaceuticals" covers discovery, development, and delivery of drug. The post-genomic era is revolutionizing the drug discovery process. The new challenges in the identification of therapeutic targets require efficient and cost-effective tools. Label-free detection systems use proteins or ligands coupled to materials, the physical properties of which are measurably modified following specific interactions. Among the label-free systems currently available, the use of metal nanoparticles offers enhanced throughput and flexibility for real-time monitoring of biomolecular recognition at a reasonable cost. This chapter will deal with the use of nanobiotechnologies for drug discovery and development, an important part of nanobiopharmaceuticals. Some technologies will accelerate target identification, whereas others will evolve into therapeutics. Use of nanobiotechnologies for drug delivery is an important part of nanomedicine.

Nanobiotechnology for Drug Discovery

Current drug discovery process needs improvement in several areas. Although many targets are being discovered through genomics and proteomics, the efficiency of screening and validation processes needs to be increased. Through further miniaturization, nanotechnology will improve the ability to fabricate massive arrays in small spaces using microfluidics and the time efficiency. This would enable direct reading of the signals from microfluidic circuits in a manner similar to a microelectronics circuit where one does not require massive instrumentation. This would increase the ability to do high-throughput drug screening. QDs and other nanoparticles (gold colloids, magnetic nanoparticles, nanobarcodes, nanobodies, dendrimers, fullerenes, and nanoshells) have received a considerable attention because of their unique properties that are useful for drug discovery (Jain 2005). Application of nanobiotechnologies to various stages of drug discovery is shown schematically in Fig. 5.1, and basic nanotechnologies applicable to drug discovery are listed in Table 5.1.

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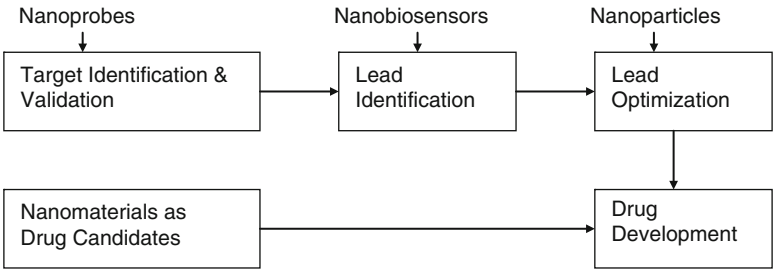

Fig. 5.1 Application of nanobiotechnology at various stages of drug discovery (© Jain PharmaBiotech)

Table 5.1 Basic nanobiotechnologies relevant to drug discovery

Nanoparticles
Gold nanoparticles
Lipoparticles
Magnetic nanoparticles
Micelles
Polymer nanoparticles
Quantum dots
Nanofibers
Nanowires
Carbon nanofibers
Nanoconduits
Nanotubes
Nanopipettes
Nanoneedles
Nanochannels
Nanopores
Nanofluidics
Nanobiotechnology applications in proteomics relevant to drug discovery
Nanoflow liquid chromatography
High-field asymmetric waveform ion mobility mass spectrometry
Use of nanotube electronic biosensor in proteomics
Fluorescence planar waveguide technology
Miscellaneous nanobiotechnologies
Visualization and manipulation at biological structures at nanoscale
Surface plasmon resonance (SPR)
Drug discovery through study of endocytosis on nanoscale
Nanosubstances as drug candidates
Dendrimers
Fullerenes
Nanobodies
Nanodevices
Nanobiosensors
Nanowire devices
Nanoarrays and nanobiochips
Cantilevers
Atomic force microscopy

Nanofluidic Devices for Drug Discovery

Development of nanofluidic devices with dimensions in the range of 1–100 nm provides opportunities for probing single molecules as fluids can no longer be considered as continua but rather as ensembles of individual molecules. Diffusion becomes an efficient mass transport mechanism on nanoscale, and these characteristics can be exploited to develop new analytical platforms for drug discovery and development.

As described in the preceding chapters, a nanopore can act as a single-molecule sensor to explore discrete molecular phenomena while operating at extremely high analytical throughput. The majority of nanopore-based studies involve the use of a protein channel that spontaneously inserts itself into a lipid membrane. A limitation of it is that it is not possible to control the pore diameter or to use it over a wide range of pH, salt concentration, temperature, and mechanical stress. An alternative to protein nanopores is the use of solid-state nanopores, which can be tuned in size with nanometer precision and display improved mechanical, chemical, and electrical stability. A novel approach for the optical detection of DNA translocation events through solid-state nanopores shows the potential for ultrahigh-throughput and parallel analysis at the single-molecule level (Chansin et al. 2007). In essence, each individual subwavelength pore acts as a waveguide for fluorescence excitation with a metallic layer on the freestanding membrane acting as an optical barrier between the illumination region and the analyte reservoir. This configuration allows for high-contrast imaging of single-molecule translocation events through multiple pores and with minimal background or noise (Hong et al. 2009).

Gold Nanoparticles for Drug Discovery

Tracking Drug Molecules in Cells

Gold nanoparticles have been used to demonstrate multiphoton-absorption-induced luminescence (MAIL), in which specific tissues or cells are fluorescently labeled using special stains that enable them to be studied. Gold nanoparticles can emit light so strongly that it is readily possible to observe a single nanoparticle at laser intensities lower than those commonly used for MAIL – sub-100-fs pulses of 790-nm light (Farrer et al. 2005). Moreover, gold nanoparticles do not blink or burn out, even after hours of observation. These findings suggest that metal nanoparticles are a viable alternative to fluorophores or semiconductor nanoparticles for biological labeling and imaging. Other advantages of the technique are that the gold nanoparticles can be prepared easily, have very low toxicity, and can readily be attached to molecules of biological interest. In addition, the laser light used to visualize the particles is a wavelength that causes only minimal damage to most biological tissues. This technology could enable tracking of a single molecule of a drug in a cell or other biological samples.

SPR with Colloidal Gold Particles

Conventional SPR is applied in specialized biosensing instruments. These instruments use expensive sensor chips of limited reuse capacity and require complex chemistry for ligand or protein immobilization. SPR has also been successfully applied with colloidal gold particles in buffered solution, which offers many advantages over conventional SPR. The support is cheap, easily synthesized, and can be coated with various proteins or protein–ligand complexes by charge adsorption. With colloidal gold, the SPR phenomenon can be monitored in any UV–vis 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.

Use of QDs for Drug Discovery

The use of QDs for drug discovery has been explored extensively. Both advantages and drawbacks have been investigated.

Advantages of the Use of QDs for Drug Discovery

- Enhanced optical properties as compared with organic dyes. QDs offer great imaging results that could not be achieved by organic dyes as they have narrowband emission together with large UV absorption spectra, which enables multiplexed imaging under a single light source.
- Multiple leads can be tested on cell culture simultaneously. Similarly, the absorption of several drug molecules can be studied simultaneously for a longer period of time.
- Using the surface functionalization properties of QDs, targeting capabilities can be added as well.
- Due to the inorganic nature of QDs, their interaction with their immediate environment at in vivo states can be minimal compared with their organic counterparts.

QDs carrying a surface-immobilized antagonist remain with nanomolar affinity on the cell surface, and particles carrying an agonist are internalized upon receptor binding. The receptor functions like a logic “and-gate” that grants cell access only to those particles that carry a receptor ligand “and” where the ligand is an agonist (Hild et al. 2010). Agonist- and antagonist-modified nanoparticles bind to several receptor molecules at a time. This multiligand binding leads to five orders of magnitude increased-receptor affinities, compared with free ligand, in displacement studies. More than 800 G protein-coupled receptors in humans provide an opportunity that targeting of a plethora of cells is possible for drug discovery and that switching from cell recognition to cell uptake is simply a matter of nanoparticle surface modification with the appropriate choice of ligand type.

Drawbacks of the Use of QDs for Drug Discovery

QDs have not been totally perfected, and some of the drawbacks are:

- Size variation during the synthesis of single color QDs is 2–4 %, which could create false results for applications such as capillary electrophoresis or gel electrophoresis. Therefore, QD synthesis techniques need to have improved quality control with respect to size distribution before they can be seriously utilized in drug discovery research.
- For ADME purposes, blue QDs (diameter of 3.7 nm) are the smallest class of the QD family, but they are considerably larger than organic dyes. Hence, the use of QDs for this purpose might not be desirable in special cases.
- Similarly, the number of functional groups attached to an organic dye is usually one, or it can be controlled very precisely. However, in the case of QDs, the functional groups usually decorate the entire surface and thus cause multiple attachments of target molecules.
- The transport of a large volume (due to multiple attachments of drug molecules to a single QD) across the membrane will be more difficult than a single molecule itself.
- To satisfy all the available surface groups, larger numbers of target molecules are needed; this could affect the cost of the experiment. Although several methods have been reported to reduce the number of surface groups around a single dot, each of these methods adds to the final size of the QDs, which might not be desired in many cases, especially in studies related to kinetics and transport of drug molecules.
- The "blinking" characteristics of QDs when they are excited with high-intensity light could be a limiting factor for fast scan systems such as flow cytometry.
- Under combined aqueous-UV excitation conditions, QDs demonstrate oxidation and release of Cd ions into the environment. This is a definite concern for in vivo applications. As an alternative, capping the surface of a core dot with a large bandgap semiconductor or proteins can eliminate or reduce the toxicity. But each additional step on the QDs will add to their final size and could even affect their final size distribution during these additional process steps.

QDs for Imaging Drug Receptors in the Brain

Cellular receptors are a critical target studied by scientists who develop new drug candidates for diseases including neurological disorders such as epilepsy and depression. More detailed understanding of the behavior of these receptors can open up new treatment options. Older imaging tools such as fluorescent dyes or polymer spheres are either too unstable or too big to effectively perform single-molecule tracking. Single-molecule properties in living cells can be tracked by using QD conjugates and produce photo resolutions up to eight times more detailed than the older imaging tools. QD conjugates are also an order of magnitude brighter than fluorescent dyes and can be observed for as long as 40 min compared to about 5 s for the dyes. Individual receptors of glycine (GlyRs), the main inhibitory

neurotransmitter in the human CNS, and their dynamics in the neuronal membrane of living cells can be studied for periods ranging from milliseconds to minutes using QDs. Entry of GlyRs into the synapse by diffusion has been observed and confirmed by electron microscopy imaging of QD-tagged receptors. Length of observation time is critical for studying cellular processes, which change rapidly over a span of several minutes.

G-protein-coupled receptors (GPCRs) are the largest protein superfamily in the human genome; they comprise 30 % of current drug targets and regulate diverse cellular signaling responses. Role of endosomal trafficking in GPCR signaling regulation is significant, but this process remains difficult to study due to the inability to distinguish among many individual receptors because of simultaneously trafficking within multiple endosomal pathways. Accurate measurement of the internalization and endosomal trafficking of single groups of serotonin (5-hydroxytryptamine, 5-HT) receptors was shown by using single QD probes and quantitative colocalization (Fichter et al. 2010). Presence of a QD tag does not interfere with 5-HT receptor internalization or endosomal recycling. Direct measurements show simultaneous trafficking of the 5-HT1A receptor in two distinct endosomal recycling pathways. Single-molecule imaging of endosomal trafficking will significantly impact the understanding of cellular signaling and provide powerful tools to elucidate the actions of GPCR-targeted therapeutics.

Lipoparticles for Drug Discovery

Lipoparticle technology (Integral Molecular Inc) 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. Lipoparticles can be paired with a multitude of detection systems, permitting the optimal detection system to be used depending on the target protein, the goal of the assay, and the preference of customers. Biosensors are one class of detection system currently being used with lipoparticles.

Biosensor for Drug Discovery with Lipoparticles

Interactions with integral membrane proteins have been particularly difficult to study because the receptors cannot be removed from the lipid membrane of a cell without disrupting the structure and function of the protein. Cell-based assays are the current standard for drug discovery against integral membrane proteins but are limited in important ways. Biosensors are capable of addressing many of these limitations. Biosensors are currently being used in target identification, validation, assay development, lead optimization, and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) studies but are best suited for soluble molecules. Integral is using lipoparticles to effectively solubilize integral membrane proteins for use in biosensors and other microfluidic devices.

A primary application of current biosensor technologies is the optimization of limited-scope drug libraries against specific targets. Paired with lipoparticle technology, biosensors can be used to address some of the most complex biological problems facing the drug discovery industry, including cell–cell recognition, cell adhesion, cell signaling, and lipid and protein–protein interactions:

- Where high-throughput screening of random libraries does not work
- Only weak ligands known, ultrasensitivity required
- When high-content information is needed (affinity, kinetics)
- Structure-based rational drug design
- ADMET: drug binding to cytochromes, serum proteins, lipid solubility
- Peptide-based ligand design where no ligand available

Lipoparticles provide a means for solubilizing integral membrane proteins that would lose their structure if extracted away from the lipid membrane. The methods for using lipoparticles range from traditional fluorescent detection technologies to emerging biosensor technologies. The optimal detection system can be used depending on the target protein, the goal of the assay, and the preference of customers.

Lipoparticles will be used for identification and optimization of chemical compounds and for antibody development. Lipoparticles will also be used to purify and concentrate structurally intact receptors from naturally occurring cell lines. This technology offers a "better-and-different" discovery platform for complex and difficult targets but can also be adapted to "faster-and-cheaper" detection systems.

Magnetic Nanoparticles Assays

Several assays are used for screening drug targets. Magnetic nanoparticles are used in many biochemical assays as labels for concentration, manipulation and, more recently, detection. Typically, one attaches the magnetic particles to the biochemical species of interest (target) using a chemically specific binding interaction. Once bound, the labels enable the manipulation of the target species through the application of magnetic forces. Spintronic sensors, specifically giant magnetoresistive and spin-dependent tunneling sensors, have been developed to detect and quantify labels in two main formats: flowing in a microfluidic channel and immobilized labels on a chip surface.

Analysis of Small Molecule–Protein Interactions by Nanowire Biosensors

Development of miniaturized devices that enable rapid and direct analysis of the specific binding of small molecules to proteins could be of substantial importance to the discovery of and screening for new drug molecules. A highly sensitive and label-free direct electrical detection of small-molecule inhibitors of ATP binding to

Abl has been reported by using silicon nanowire field-effect transistor devices (Wang et al. 2005a). Abl, which is a protein tyrosine kinase whose constitutive activity is responsible for chronic myelogenous leukemia, was covalently linked to the surfaces of silicon nanowires within microfluidic channels to create active electrical devices. Concentration-dependent binding of ATP and concentration-dependent inhibition of ATP binding by the competitive small-molecule antagonist Gleevec were assessed by monitoring the nanowire conductance. In addition, concentration-dependent inhibition of ATP binding was examined for four additional small molecules, including reported and previously unreported inhibitors. These studies demonstrate that the silicon nanowire devices can readily and rapidly distinguish the affinities of distinct small-molecule inhibitors and, thus, could serve as a technology platform for drug discovery.

Cells Targeting by Nanoparticles with Attached Small Molecules

Multivalent attachment of small molecules to nanoparticles can increase specific binding affinity and reveal new biological properties of such nanomaterial. Multivalent drug design has yielded antiviral and anti-inflammatory agents several orders of magnitude more potent than monovalent agents. Parallel synthesis of a library has been described, which is comprised of nanoparticles decorated with different synthetic small molecules (Weissleder et al. 2005). Screening of this library against different cell lines led to discovery of a series of nanoparticles with high specificity for endothelial cells, activated human macrophages, and pancreatic cancer cells. This multivalent approach could facilitate development of functional nanomaterials for applications such as differentiating cell lines, detecting distinct cellular states, and targeting specific cell types. It has potential applications in high-throughput drug discovery, diagnostics, and human therapeutics.

Role of AFM for Study of Biomolecular Interactions for Drug Discovery

An approach called TREC (topography and recognition imaging) uses any of a number of different ligands such as antibodies, small organic molecules, and nucleotides bound to a carefully designed AFM tip sensor which can, in a series of unbinding experiments, estimate affinity and structural data (Ebner et al. 2005). 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 under a wide range of circumstances. By functionalizing the tip, one can use it to probe biological systems and identify particular chemical entities on the surface of a biological sample. This opens the door to more effective use 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 a protein's physical state, called "conformational" diseases, it is particularly well suited for study with AFM. Extensive data suggest that the conversion of the Aβ peptide from soluble to insoluble forms is a key factor in the pathogenesis of Alzheimer's disease (AD). In recent years, AFM has provided useful insights into the physicochemical processes involving Aβ morphology. AFM was the key in identifying the nanostructures which are now recognized as different stages of Aβ aggregation in AD and has revealed other forms of aggregation, which are observable at earlier stages and evolve to associate into mature fibrils. AFM can now be used to explore factors that either inhibit or promote fibrillogenesis, e.g., AFM can be used to compare monoclonal antibodies being studied as potential treatments for AD to select the one that does a better job of inhibiting the formation of these protofibrils. AFM not only can be reliably used to study the effect of different molecules on Aβ aggregation but it can also provide additional information such as the role of epitope specificity of antibodies as potential inhibitors of fibril formation.

Nanoscale Devices for Drug Discovery

Miniature devices are being used to study synthetic cell membranes in an effort to speed the discovery of new drugs for a variety of diseases, including cancer. Examples of these are "laboratories-on-a-chip" and Lab-on-Bead.

Laboratories-on-a-Chip

Microfluidic systems and nanoporous materials enable construction of miniature "laboratories-on-a-chip" that might contain up to a million test chambers, each capable of screening an individual drug. Such chips can be used to screen candidate compounds to find drugs to overcome anticancer drug resistance by deactivating the pumps in cell membranes that remove chemotherapy drugs from tumor cells, making the treatment less effective. The chips could dramatically increase the number of experiments that are possible with a small amount of protein.

Lab-on-Bead

A nanotechnology-based method for selecting peptide nucleic acid (PNA)-encoded molecules with specific functional properties from combinatorially generated libraries consists of three essential stages: (1) creation of a Lab-on-Bead library, a one-bead, one-sequence library that, in turn, displays a library of candidate molecules; (2) fluorescence microscopy-aided identification of single target-bound beads and the extraction – wet or dry – of these beads and their attached candidate molecules by a micropipette manipulator; and (3) identification of the target-binding

candidate molecules via amplification and sequencing (Gassman et al. 2010). This novel integration of techniques harnesses the sensitivity of DNA detection methods and the multiplexed and miniaturized nature of molecule screening to efficiently select and identify target-binding molecules from large nucleic acid-encoded chemical libraries and has the potential to accelerate assays currently used for the discovery of new drug candidates by screening millions of chemicals simultaneously using nanosized plastic beads. One batch of nanoscopic beads can replace the work of thousands of conventional, repetitive laboratory tests. This process could be up to 10,000 times faster than current methods. By working at nanoscale, it will be possible to screen more than a billion possible drug candidates per day as compared to the current limit of hundreds of thousands per day.

Nanotechnology for Drug Design at Cellular Level

To create drugs capable of targeting some of the most devastating human diseases, one must first decode exactly how a cell or a group of cells communicates with other cells and reacts to a broad spectrum of complex biomolecules surrounding it. But even the most sophisticated tools currently used for studying cell communications suffer from significant deficiencies and typically can only detect a narrowly selected group of small molecules or, for a more sophisticated analysis, the cells must be destroyed for sample preparation. A nanoscale probe, the scanning mass spectrometry (SMS) probe, can capture both the biochemical makeup and topography of complex biological objects. SMS exploits an approach to electrospray ionization that enables continuous sampling from a highly localized picoliter volume in a liquid environment, softly ionizes molecules in the sample to render them amenable for mass spectrometric analysis, and sends the ions to the mass spectrometer (Kottke et al. 2010). The SMS probe can help map all those complex and intricate cellular communication pathways by probing cell activities in the natural cellular environment, which might lead to better disease diagnosis and drug design on the cellular level.

Nanobiotechnology-Based Drug Development

Dendrimers as Drugs

Dendrimers are a novel class of three-dimensional nanoscale, core–shell structures that can be precisely synthesized for a wide range of applications. Specialized chemistry techniques allow for precise control over the physical and chemical properties of the dendrimers. They are most 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. Polymer–protein and polymer–drug conjugates can be developed as anticancer drugs. These have the following advantages:

- Tailor-made surface chemistry
- Nonimmunogenic
- Inherent body distribution enabling appropriate tissue targeting
- Possibly biodegradable

Dendrimer conjugation with low molecular weight drugs has been of increasing interest recently for improving pharmacokinetics, targeting drugs to specific sites, and facilitating cellular uptake. Opportunities for increasing the performance of relatively large therapeutic proteins such as streptokinase (SK) using dendrimers have been explored in one study (Wang et al. 2007a). Using the active ester method, a series of streptokinase-poly(amido amine) (PAMAM) G3.5 conjugates were synthesized with varying amounts of dendrimer-to-protein molar ratios. All of the SK conjugates displayed significantly improved stability in phosphate buffer solution, compared to free SK. The high coupling reaction efficiencies and the resulting high enzymatic activity retention achieved in this study could enable a desirable way for modifying many bioactive macromolecules with dendrimers.

Glycodendrimers are carbohydrate-functionalized dendrimers for use in therapeutics, antigen presentation, and as biologically active compounds. GlycoSyn, a joint venture between Starpharma Holdings and Industrial Research Ltd, will provide manufacturing and specialized expertise in carbohydrate design, synthesis, and analysis. One of the first projects in the pipeline involves research undertaking cGMP manufacture of intermediates used in the production of Starpharma's vaginal microbicide – VivaGel, a polyvalent dendrimer-based pharmaceutical being developed to prevent the spread of HIV/AIDS and potentially other sexually transmitted infections including genital herpes.

Fullerenes as Drug Candidates

A key attribute of the fullerene molecules is their 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 for positional control in matching fullerene compounds to biological targets. In concert with other attributes, namely, the size of the fullerene molecules, their redox potential, and its relative inertness in biological systems, it is possible to tailor requisite pharmacokinetic characteristics to fullerene-based compounds and optimize their therapeutic effect.

Fullerene antioxidants bind and inactivate multiple circulating intracellular free radicals, giving them unusual power to stop free radical injury and to halt the progression of diseases caused by excess free radical production. Fullerenes provide

effective defense against all of the principal damaging forms of reactive oxygen species. C-60 fullerene has 30 conjugated carbon–carbon double bonds, all of which can react with a radical species. In addition, the capture of radicals by fullerenes is too fast to measure and is referred to as "diffusion controlled," meaning the fullerene forms a bond with a radical every time it encounters one. Numerous studies demonstrate that fullerene antioxidants work significantly better as therapeutic antioxidants than other natural and synthetic antioxidants, at least for CNS degenerative diseases. In oxidative injury or disease, fullerene antioxidants can enter cells and modulate free radical levels, thereby substantially reducing or preventing permanent cell injury and cell death. Mechanisms of action of fullerene are as follows:

- Fullerenes can capture multiple electrons derived from oxygen free radicals in unoccupied orbitals.
- When an attacking radical forms a bond with fullerene creating 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.

Fullerenes have potential applications in the treatment of diseases where oxidative stress plays a role in the pathogenesis. These include the following:

- Degenerative diseases of the central nervous system including Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis
- Multiple sclerosis
- Ischemic cardiovascular diseases
- Atherosclerosis
- Major long-term complications of diabetes
- Sun-induced skin damage and physical manifestations of aging

The first-generation antioxidant fullerenes are based on the C3 compound, produced by the precise grafting of three malonic acid groups to the C-60 fullerene surface. C3 has shown significant activity against a spectrum of neurodegenerative disorders in animal models. These animal models replicate many of the features of important human neurodegenerative diseases, including amyotrophic lateral sclerosis and Parkinson's disease.

The second-generation antioxidant fullerenes are based on DF-1, the dendrofullerene, produced by attaching a highly water-soluble conjugate to the C-60 fullerene core. In preclinical testing, C-60 has shown DF-1 to be highly soluble, nontoxic, and able to retain a high level of antioxidant activity in both cultured cells and animals.

A number of water-soluble C60 derivatives have been suggested for various medical applications. These applications include neuroprotective agents, HIV-1

protease inhibitors, bone disorder drugs, transfection vectors, X-ray contrast agents, photodynamic therapy agents, and a C60–paclitaxel chemotherapeutic.

Another possible application of fullerenes is to be found in nuclear medicine, in which they could be used 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 effect profile because fullerenes are resistant to biochemical degradation within the body.

Nanobodies

Nanobodies, derived from naturally occurring single-chain antibodies, are the smallest fragments of naturally occurring heavy-chain antibodies that have evolved to be fully functional in the absence of a light chain. The Nanobody technology (Ablynx) was originally developed following the discovery that camelidae (camels and llamas) possess a unique repertoire of fully functional antibodies that lack light chains. Like conventional antibodies, nanobodies show high target specificity and low inherent toxicity; however, like small-molecule drugs, they can inhibit enzymes and can access receptor clefts. Their unique structure consists of a single variable domain (VHH), a hinge region, and two constant domains (CH2 and CH3). The cloned and isolated VHH domain is a perfectly stable polypeptide harboring the full antigen-binding capacity of the original heavy chain. This newly discovered VHH domain is the basic component of Ablynx's Nanobodies. Ablynx's Nanobodies are naturally highly homologous to human antibodies. They can also be humanized to within 99 % sequence homology of human VH domains. Ablynx's Nanobody platform can quickly deliver therapeutic leads for a wide range of targets. Advantages of nanobodies are:

- They combine the advantages of conventional antibodies with important features of small-molecule drugs.
- Nanobodies can address therapeutic targets not easily recognized by conventional antibodies such as active sites of enzymes.
- Nanobodies are very stable.
- They can be administered by means other than injection.
- They can be produced cost-effectively on a large scale.
- Nanobodies have an extremely low immunogenic potential. In animal studies, the administration of nanobodies does not yield any detectable humoral or cellular immune response.

The cloning and selection of antigen-specific nanobodies obviate the need for construction and screening of large libraries and for lengthy and unpredictable in vitro affinity maturation steps. The unique and well-characterized properties enable nanobodies to excel conventional therapeutic antibodies in terms of recognizing

uncommon or hidden epitopes, binding into cavities or active sites of protein targets, tailoring of half-life, drug format flexibility, low immunogenic potential, and ease of manufacture. Moreover, the favorable biophysical and pharmacological properties of nanobodies, together with the ease of formatting them into multifunctional protein therapeutics, leave them ideally placed as a new generation of antibody-based therapeutics. They have a potential as cancer therapeutic agents.

Another example of use of nanobodies as novel drugs is nanobody-conjugated human trypanolytic factor for treatment of human African trypanosomiasis (HAT). Normal human serum (NHS) contains apolipoprotein L-I (apoL-I), which lyses African trypanosomes except resistant forms such as *Trypanosoma brucei rhodesiense*, which expresses the apoL-I-neutralizing serum resistance-associated (SRA) protein, endowing this parasite with the ability to infect humans and cause HAT. A truncated apoL-I (Tr-apoL-I) has been engineered by deleting its SRA-interacting domain, which makes it lytic for *T. b. rhodesiense*. Tr-apoL-I has been conjugated with a nanobody that efficiently targets conserved cryptic epitopes of the variant surface glycoprotein of trypanosomes to generate a new type of immunotoxin with potential for trypanosomiasis therapy (Baral et al. 2006). Treatment with this engineered conjugate resulted in clear curative and alleviating effects on acute and chronic infections of mice with both NHS-resistant and NHS-sensitive trypanosomes. First-in-class' antithrombotic agent ALX-0081 (Ablynx NV) is in phase I trials (Van Bockstaele et al. 2009).

Role of Nanobiotechnology in the Future of Drug Discovery

None of the nanoparticles available is ideal for all requirements of drug discovery. The choice may depend on the 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, there are some limitations yet to be resolved for their use in the drug discovery studies, namely, toxicity, size variation, agglomeration, potential multiple drug attachment to a single QD, and blinking.

An increasing use of nanobiotechnology by the pharmaceutical and biotechnology industries is anticipated. Nanotechnology will be applied at all stages of drug development – from formulations for optimal delivery to diagnostic applications in clinical trials. In the near future, it may be possible to fully model an individual cell's structure and function by 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 any experiments in living animals. In another promising area of application, non-biodegradable 3D scaffolds are being developed to hold stem cells for pharmaceutical and biological research. These tissue constructs can be used to test new drugs. Since tissues grow in three dimensions and not two, 3D growth would be more suitable for early drug screening.

Nanobiotechnology in Drug Delivery

Drug delivery is one of the important considerations in drug development and therapeutics. New technologies are applied for constructing innovative formulations and delivering them. The focus is on targeted drug delivery. This is important for delivery of biopharmaceuticals and treatment of diseases such as cancer and neurological disorders. In the pharmaceutical industry, there is potential to provide new formulations and routes of drug delivery. Among new technologies, nanobiotechnology has evoked considerable interest for application in the pharmaceutical industry. Applications of nanotechnology for drug delivery will be considered in this chapter.

Ideal Properties of Material for Drug Delivery

Properties of an ideal macromolecular drug delivery or biomedical vector are:

- Structural control over size and shape of drug or imaging-agent cargo-space
- Biocompatible, nontoxic polymer/pendant functionality
- Well-defined scaffolding and/or surface modifiable functionality for cell-specific targeting moieties
- Lack of immunogenicity or ability to evade the immune system
- Appropriate cellular adhesion, endocytosis, and intracellular trafficking to allow therapeutic delivery or imaging in the cytoplasm or nucleus
- Acceptable bioelimination or biodegradation
- Targeted delivery with binding to the target sites and accumulation in the target tissue with sparing of normal or nontarget tissues
- Controlled or triggerable drug release
- Molecular level isolation and protection of the drug against inactivation during transit to target cells
- Minimal nonspecific cellular and blood–protein binding properties
- Ease of consistent, reproducible, clinical grade synthesis

Nanobiotechnology fulfills many of these requirements for improved drug delivery. Nanoparticles as well as nanodevices are used for this purpose.

Improved Absorption of Drugs in Nanoparticulate Form

Micronization was in use prior to introduction of techniques for producing nanoparticles. Although several claims were made for increased absorption, no significant improvement was documented because the microparticle size was still above 3 μm (3,000 nm) and nanoparticle size could be as much as 30 times less.

Reduction of particle size from 5 μm to 200 nm increases the surface area of the particle by a factor of 25 with increase in solubility. As an example, reduction of iron phosphate to the nanoscale increases its absorption in the body.

Interaction of Nanoparticles with Human Blood

Nanoparticle size and plasma binding profile contribute to a particle's longevity in the bloodstream, which can have important consequences for therapeutic efficacy. Approximate doubling in nanoparticle hydrodynamic size was observed upon in vitro incubation of 30–50-nm colloidal gold nanoparticles in human plasma due to binding of plasma proteins to their surface (Dobrovolskaia et al. 2009).

Nanoscale Devices Delivery of Therapeutics

There are several requirements for developing a device small enough to efficiently leave the vasculature and enter cells to perform multiple, smart tasks. However, the major requirement involves size. Vascular pores limit egress of therapeutics to materials less than approximately 50 nm in diameter, and cells will not internalize materials much greater than 100 nm. As a result, the only currently available technology that fulfills these criteria consists of synthetic nanodevices. These are designed synthetic materials with structures less than 100 nm in size. Unlike fictional mechanical nanomachines, based on machines that have been "shrunken" to nanometer dimensions, several true nanomolecular structures have now been synthesized and applied to drug delivery, gene transfer, antimicrobial therapeutics, and immunodiagnostics.

Nanobiotechnology Solutions to the Problems of Drug Delivery

One of the major problems with drugs is solubility, which is an essential factor for drug effectiveness, independent of administration route. It is also a major challenge for pharmaceutical companies developing new pharmaceutical products since nearly half of new chemically based drugs are insoluble, or poorly soluble, in water. Many, otherwise promising, compounds never reach the market. Others reach the market but in a suboptimal formulation, possibly providing low or unpredictable bioavailability or posing an increased side effect risk. Enhanced solubility technology can be used to reformulate such drugs and increase their commercial potential. Nanobiotechnology provides the following solutions to the problems of drug delivery:

- The particle size is reduced to nanometer size range to increase the surface area, thereby increasing the rate of dissolution, e.g., Nanoedge technology (Baxter).
- Improving solubilization of the drug.

- Using noninvasive routes of administration eliminates the need for administration of drugs by injection.
- Development of novel nanoparticle formulations with improved stabilities and shelf lives.
- Development of nanoparticle formulations for improved absorption of insoluble compounds and macromolecules enables improved bioavailability and release rates, potentially reducing the amount of dose required and increasing safety through reduced side effects.
- Manufacture of nanoparticle formulations with controlled particle sizes, morphology, and surface properties would be more effective and less expensive than other technologies.
- Nanoparticle formulations that can provide sustained-release profiles up to 24 h can improve patient compliance with drug regimens.
- Direct coupling of drugs to targeting ligand restricts the coupling capacity to a few drug molecules, but coupling of drug carrier nanosystems to ligands allows import of thousands of drug molecules by means of one receptor-targeted ligand. Nanosystems offer opportunities to couple drugs with newly discovered disease-specific targets.

Nanosuspension Formulations

Nanosuspension formulations can be used to improve solubility of poorly soluble drugs. A large number of new drug candidates emerging from drug discovery programs are water insoluble, and therefore poorly bioavailable, leading to abandoned development efforts. These can now be rescued by formulating them into crystalline nanosuspensions. Techniques such as media milling and high-pressure homogenization have been used commercially for producing nanosuspensions. The unique features of nanosuspensions have enabled their use in various dosage forms, including specialized delivery systems such as mucoadhesive hydrogels. Nanosuspensions can be delivered by parenteral, peroral, ocular, and pulmonary routes. Currently, efforts are being directed to extending their applications in site-specific drug delivery. A large number of drugs are available as nanosuspensions. Advantages of nanosuspension are (Patel and Agrawal 2011):

- Higher drug loading can be achieved.
- Dose reduction is possible.
- Enhancement of physical and chemical stability of drugs.
- Suitable for hydrophilic drugs.

Baxter scientists have used Nanoedge technology to formulate the antifungal agent itraconazole as an intravenous nanosuspension. In studies on rats, formulation as a nanosuspension was shown to enhance efficacy of itraconazole relative to a solution formulation because of altered pharmacokinetics, leading to increased tolerability, permitting higher dosing and resultant tissue drug levels (Rabinow et al. 2007).

A study has compared the in vitro and in vivo antitumor efficacy as well as dose-dependent toxicity of camptothecin nanosuspension (Nano-CPT) with that of topotecan (TPT). Nano-CPT showed approximately six times in vitro cytotoxicity than TPT against cell lines MCF-7, and the same in vivo antitumor activity as TPT but with lower toxicity (Yao et al. 2012). The results indicate that Nano-CPT formulation has higher antitumor efficacy and lower toxicity than the conventional formulation of the drug.

Nanotechnology for Solubilization of Water-Insoluble Drugs

The Ubisol-Aqua™ (Zymes LLC) delivery system uses nanotechnology to enable the solubilization and reformulation of water-insoluble drugs, nutrients, and cosmetic ingredients. The capacity of Ubisol-Aqua™ to expand the usefulness of such compounds has been scientifically demonstrated with water-soluble formulations of coenzyme Q10 (HQO™) and antifungal antibiotics. Zymes LLC has successfully solubilized fish oil and omega-3 fatty acids (DHA/EPA/ALA) with an average particle size of 34 nm. Zymes offers its delivery system technology to industry partners in need of more effective ways of making their ingredients water soluble and thus more bioavailable.

Self-Assembled Nanostructures with Hydrogels for Drug Delivery

Drug delivery systems based on physical hydrogels with self-assembled nanostructures are attracting increasing attention as complements to chemically cross-linked hydrogels because of advantages of reduced toxicity, convenience of in situ gel formation, stimuli responsiveness, reversible sol-gel transition, and improved drug loading and delivery profiles. The driving forces of the self-assembly include hydrophobic interaction, hydrogen bonding, electrostatic interaction, and weak van der Waals forces. Stimuli-responsive properties of physical hydrogels include thermosensitivity and pH sensitivity. Fabrication of self-assembled nanostructures in drug delivery hydrogels, via physical interactions between polymer–polymer and polymer–drug, requires accurately controlled macro- or small molecular architecture and a comprehensive knowledge of the physicochemical properties of the therapeutics (Tang et al. 2011). Nanostructures within hydrogels, which interact with payloads, provide useful means to stabilize the drug form and control its release kinetics. Biocompatibles UK Ltd, a subsidiary of BTG plc, is developing these systems.

Nanomaterials and Nanobiotechnologies Used for Drug Delivery

Table 5.2 shows various nanomaterials and nanobiotechnologies used for drug delivery.

Table 5.2 Nanomaterials used for drug delivery

Structure	Size	Role in drug delivery
Bacteriophage NK97 (a virus that attacks bacteria)	~28 nm	Emptied of its own genetic material, NK97, which is covered by 72 interlocking protein rings, can act as a nanocontainer to carry drugs and chemicals to targeted locations
Canine parvovirus (CPV)-like particles	~26 nm	Targeted drug delivery: CPV binds to transferrin receptors, which are overexpressed in some tumors
Carbon magnetic nanoparticles	40–50 nm	For drug delivery and targeted cell destruction
Ceramics nanoparticles	~35 nm	Accumulate exclusively in the tumor tissue and allow the drug to act as sensitizer for PDT without being released
Cerasomes	60–200 nm	Cerasome is filled with C6 ceramide for use as an anticancer agent
Dendrimers	1–20 nm	Holding therapeutic substances such as DNA in their cavities
Gold nanoparticles	2–4 nm	Enable externally controlled drug release
HTCC nanoparticles	110–180 nm	Encapsulation efficiency is up to 90 %. In vitro release studies show a burst effect followed by a slow and continuous release
Micelle/nanopill	25–200 nm	Made from two polymer molecules – one water repellant and the other hydrophobic – that self-assemble into a sphere called a micelle that can deliver drugs to specific structures within the cell
Low-density lipoproteins	20–25 nm	Drugs solubilized in the lipid core or attached to the surface
Nanocochleates		Nanocochleates facilitate delivery of biologicals such as DNA and genes
Nanocrystals	<1,000 nm	NanoCrystal technology (Elan) can rescue a significant number of poorly soluble chemical compounds by increasing solubility
Nanodiamonds	550–800 nm	Biocompatibility and unique surface properties for drug delivery
Nanoemulsions	20–25 nm	Drugs in oil and/or liquid phases to improve absorption
Nanoliposomes	25–50 nm	Incorporate fullerenes to deliver drugs that are not water soluble and tend to have large molecules
Nanoparticle composites	~40 nm	Attached to guiding molecules such as MAbs for targeted drug delivery
Nanopore membrane		An implanted titanium device using silicone nanopore membrane can release encapsulated protein and peptide drugs
Nanospheres	50–500 nm	Hollow ceramic nanospheres created by ultrasound
Nanostructured organogels	~50 nm	Mixture of olive oil, liquid solvents, and adding a simple enzyme to chemically activate a sugar. Used to encapsulate drugs
Nanotubes	Single wall 1–2 nm Multiwall 20–60 nm	Resemble tiny drinking straws and that might offer advantages over spherical nanoparticles for some applications
Nanovalve	~500 nm	Externally controlled release of drug into a cell
Nanovesicles	25–100 nm	Bilayer spheres containing the drugs in lipids
Polymer nanocapsules	50–200 nm	Enclosing drugs
PEG-coated PLA nanoparticles	Variable size	PEG coating improves the stability of PLA nanoparticles in the gastrointestinal fluids and helps the transport of encapsulated protein across the intestinal and nasal mucus membranes
Quantum dots	2–10 nm	Combine imaging with therapeutics
Superparamagnetic iron oxide nanoparticles	10–100 nm	As drug carriers for intravenous injection to evade RES as well as to penetrate the very small capillaries within the body tissues and thus offer the most effective distribution in certain tissues

PEG poly(ethylene glycol), *PLA* poly(lactic acid), *HTCC* N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride, *RES* reticuloendothelial system

Viruses as Nanomaterials for Drug Delivery

Specific targeting of tumor cells is an important goal for the design of nanotherapeutics for the treatment of cancer. Recently, viruses have been explored as nanocontainers for specific targeting applications, but these systems typically require modification of the virus surface using chemical or genetic means to achieve tumor-specific delivery. However, there is a subset of viruses with natural affinity for receptors on tumor cells that could be exploited for nanotechnology applications, e.g., the canine parvovirus for targeted drug delivery in cancer.

Bacteria-Mediated Delivery of Nanoparticles and Drugs into Cells

Nanoparticles and bacteria have been independently used to deliver genes and proteins into mammalian cells for monitoring or altering gene expression and protein production. Harmless strains of bacteria could be used as vehicles, harnessing bacteria's natural ability to penetrate cells and their nuclei. Researchers at Purdue University's Birck Nanotechnology Center have demonstrated the simultaneous use of nanoparticles and bacteria to deliver nucleic acid-based model drug molecules into cells in mice (Akin et al. 2007). In this approach, the gene or cargo is loaded onto the nanoparticles, ranging in size from 40 to 200 nm, which are attached to the bacteria with linker molecules. The bacteria successfully deliver the molecules, and the genes are released from the nanoparticles and expressed in cells. When the cargo-carrying bacteria attach to the recipient cell, they are engulfed by its outer membrane, forming "vesicles" or tiny spheres that are drawn into the cell's interior. Once inside the cell, the bacteria dissolve the vesicle membrane and release the cargo as shown in Fig. 5.2.

This technique may be used to deliver different types of cargo into a variety of cells and live animals for gene therapy without the need for complicated genetic manipulations. This delivery system also is more efficient than techniques using viruses as they usually incorporate only one copy of a gene cargo to virus particle. In this approach, bacteria can carry hundreds of nanoparticles, each of which can in turn carry hundreds of drug molecules, depending on the size of the nanoparticles. Released cargo can be designed to be transported to different locations in the cells to carry out disease detection and treatment simultaneously. The method might be used to take images of diseased tissues by inserting a cargo of fluorescent molecules into tumors that are ordinarily too small to be detected. It could enable insertion of relatively large structures, such as biosensors into the interiors of cells for the early detection of cancer and other diseases and to monitor the progress of disease as well as response to drug therapy. The carbon nanotubes could be delivered into diseased cells and then exposed to light, causing them to heat up and selectively kill only the diseased cells.

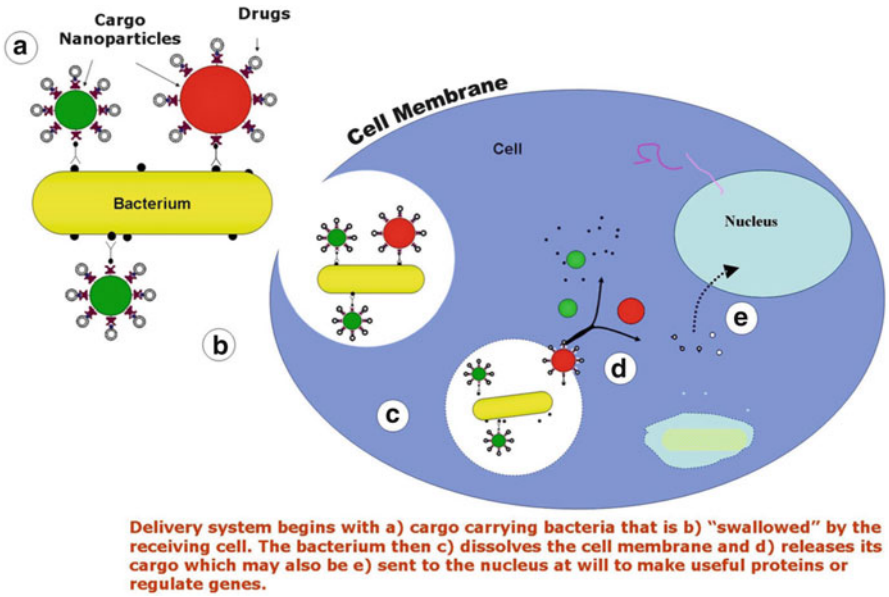

Fig. 5.2 Bacteria plus nanoparticles for drug delivery into cells (Source: Akin et al. 2007)

Cell-Penetrating Peptides

Cell-penetrating peptides (CPPs) are short basic peptide sequences that might display amphipathic properties. CPPs generally contain a small number (<20–30) of amino acids, among which are a great number of positively charged amino acids that confer cell internalization properties on those peptides. Originally derived from natural proteins, the number of designed CPPs with similar cell penetration properties has now expanded widely. These positively charged peptides internalize into all cell types, but with different efficiency. CPPs use all routes of pinocytosis to internalize, in addition to direct membrane translocation that requires interaction with lipid membrane domains. These differences in internalization efficiency according to the peptide sequence and cell type suggest that the CPPs interact with different molecular partners at the cell surface. The most popular CPPs are penetratin, Tat, and oligoarginine, which interact with carbohydrates and lipids. Cell surface composition influences cell internalization, and the interaction with molecules found in membranes reflects the internalization efficiency of the peptides (Walrant et al. 2012). For specific drug delivery, the exact molecular and chemical nature of membranes and their interactions with CPPs need to be identified.

Nanoparticle-Based Drug Delivery

Trend toward miniaturization of carrier particles had already started prior to the introduction of nanotechnology in drug delivery. As a part of introduction, microparticles and nanoparticles will be compared for their role as carriers of therapeutic substances

The suitability of nanoparticles for use in drug delivery depends on a variety of characteristics, including size and porosity. Acusphere Inc is creating porous particles that are smaller than red blood cells. Nanoparticles can be used to deliver drugs to patients through various routes of delivery. Nanoparticles are important for delivering drugs intravenously so that they can pass safely through the body's smallest blood vessels, for increasing the surface area of a drug so that it will dissolve more rapidly, and for delivering drugs via inhalation. Porosity is important for entrapping gases in nanoparticles, for controlling the release rate of the drug and for targeting drugs to specific regions.

It is difficult to create sustained-release formulations for many hydrophobic drugs because they release too slowly from the nanoparticles used to deliver the drug, diminishing the efficacy of the delivery system. Modifying water uptake into the nanoparticles can speed the release while retaining the desired sustained-release profile of these drugs. Water uptake into nanoparticles can be modified by adjusting the porosity of the nanoparticles during manufacturing and by choosing from a wide variety of materials to include in the shell. Different types of nanoparticles and nanotechnologies used for drug delivery will be mentioned briefly here, and specialized drug delivery for various disorders will be described in chapters dealing with those disorders.

Cationic Nanoparticles

Cationic nanoparticles built from drug, cationic lipid, and polyelectrolytes are excellent and active carriers of amphotericin B against *Candida albicans* (Vieira and Carmona-Ribeiro 2008). Assemblies of amphotericin B and cationic lipid, at extreme drug to lipid molar ratios, were wrapped by polyelectrolytes forming cationic nanoparticles of high colloid stability and fungicidal activity against *C. albicans*. Experimental strategy involved dynamic light scattering for particle sizing, zeta-potential analysis, determination of colloid stability, determination of AmB aggregation state by optical spectra, and determination of activity against *C. albicans* in vitro from cfu countings. The multiple assembly of antibiotic, cationic lipid and cationic polyelectrolyte consecutively nanostructured in each particle produced a strategical and effective attack against fungal infections.

Ceramic Nanoparticles

Ceramic (inorganic) particles with entrapped biomolecules have potential pharmaceutical applications in including drug delivery. Ceramic nanoparticles have several advantages such as:

- Manufacture processes are relatively similar to the well-known sol-gel process, require ambient temperature condition, and can be easily prepared with the desired size, shape, and porosity.
- Their small size (less than 50 nm) can help them to evade being trapped by the reticuloendothelial system of the body.
- There is no swelling or change in porosity with change in pH.
- These particles effectively protect doped molecules (enzymes, drugs, etc.) against denaturation induced by external pH and temperature.
- Such particles, including silica, aluminum, and titanium, are known for their compatibility with biological systems.
- Their surfaces can be easily modified for conjugation to monoclonal antibodies or ligands to target them to desired sites in vivo.

Cyclodextrin Nanoparticles for Drug Delivery

Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic center. Cyclodextrin molecules are relatively large with a number of hydrogen donors and acceptors and, thus, in general they do not permeate lipophilic membranes. Cyclodextrins have mainly been used as complexing agents to increase aqueous solubility of poorly soluble drugs and to increase their bioavailability and stability.

Amphiphilic cyclodextrin nanoparticles, resulting from the esterification of primary hydroxyl groups by hydrocarbon chains varying from C6 to C14, are capable of forming spontaneously nanoparticles, which can be loaded with drugs. The drug can be released in a controlled manner following targeting delivery by the oral or the parenteral route. For injectable preparations, sterile filtration is not feasible since nanoparticle sizes are larger than the filter pore size and the yield after sterilization is very low. However, blank as well as drug-loaded cyclodextrin nanospheres and nanocapsules are capable of being sterilized by gamma irradiation with no effect on particle size, drug loading, and drug release properties.

Incorporation of cyclodextrins in poly(anhydride) nanoparticles improves their bioadhesive capability, the loading of lipophilic drugs, and has an effect on efflux membrane proteins as well as cytochrome P450. The combination between bioadhesive nanoparticles and P-gp inhibitors without pharmacological activity may be useful for promoting the oral bioavailability of drugs (Agüeros et al. 2011). CALAA01 (Calando Pharmaceuticals), a targeted, self-assembling nanoparticle system based on cyclodextrin complexed with siRNA, overcomes delivery problems of siRNAs and has been shown to be effective in phase I clinical trials.

Dendrimers for Drug Delivery

Well-characterized, commercially available dendritic polymers have been subjected to functionalization for preparing drug delivery systems of low toxicity, high loading capacity, ability to target specific cells, and transport through their membranes. This has been achieved by surface targeting ligands, which render the carriers specific to certain cells and PEG, securing water solubility, stability, and prolonged circulation. Moreover, transport agents facilitate transport through cell membranes, while fluorescent probes detect their intracellular localization. A common feature of surface groups is multivalency, which considerably enhances their binding strength with complementary cell receptors. To these properties, one should also add the property of attaining high loading of active ingredients coupled with controlled and/or triggered release (Paleos et al. 2010).

The unique properties of dendrimers such as high degree of branching and well-defined molecular weight make them ideal scaffolds for drug delivery. Advantages of dendrimers over linear polymers are:

- The large number of active functional groups on the surface of dendrimers allows them to be meticulously tailored and to act as nanoscaffolds or nanocontainers for various categories of drugs (Jain and Asthana 2007).
- Because of their well-defined molecular weight, they provide reproducible pharmacokinetic behavior compared to linear polymers containing fractions within a sample that vary greatly in molecular weight.
- The globular structure of dendrimers, as contrasted with the coil structure of most linear polymers, can modify their biological properties, enabling discovery of new effects related to macromolecular architecture.

Dendrimers are particularly useful for the delivery of anticancer drugs such as cisplatin and doxorubicin. Dendrimers are also agents for boron neutron capture therapy and photodynamic therapy for cancer. By adding stimuli-responsive properties to the dendrimers, dendritic polymers capable of controlled release can be produced (Kojima 2010). These stimuli-responsive dendrimers are potential next-generation drug carriers.

DNA-Assembled Dendrimers for Drug Delivery

A wide variety of nanoparticle drug delivery systems have been developed using DNA molecules to bind the dendrimers together. Nanometer-scaled dendrimers can be assembled in many configurations by using attached lengths of ssDNA molecules, which naturally bind to other DNA strands in a highly specific fashion. This approach enables targeting of a wide variety of molecules – drugs, contrast agents – to almost any cell. Nanoparticle complexes can be specifically targeted to cancer cells and are small enough to enter a diseased cell, either killing it from within or sending out a signal to identify it. However, construction of the particles is difficult and time-consuming.

Fullerenes for Drug Delivery

Amphiphilic Fullerene Derivatives

The amphiphilic fullerene monomer (AF-1) consists of a buckyball cage to which a Newkome-like dendrimer unit and five lipophilic C12 chains positioned octahedrally to the dendrimer unit are attached. An AF-1-based liposome termed "buckysome" has been described that is water soluble and forms stable spherical nanometer sized vesicles (Partha et al. 2007). Cryogenic electron microscopy (cryo-EM) indicates the formation of large (400 nm diameter) multilamellar, liposome-like vesicles and unilamellar vesicles in the size range of 50–150-nm diameter. In addition, complex networks of cylindrical, tube-like aggregates with varying lengths and packing densities were observed. Under controlled experimental conditions, high concentrations of spherical vesicles could be formed. In vitro results suggest that these supramolecular structures impose little to no toxicity. Ongoing studies are aimed at understanding cellular internalization of these nanoparticle aggregates. This delivery vector might provide promising features such as ease of preparation, long-term stability, and controlled release.

Fullerene Conjugates for Intracellular Delivery of Peptides

Cell walls, or membranes, form a protective covering around the cell's inner machinery and its DNA blueprints. Drugs are far more effective if they are delivered through the membrane directly into the cell, but this is difficult. A fullerene–peptide conjugate formed via the incorporation of a fullerene-substituted phenylalanine derivative, "bucky amino acid" (Baa), to a cationic peptide, acts as a passport for intracellular delivery, enabling transport of peptides that, in the absence of the fullerene amino acid, cannot enter the cell (Yang et al. 2007). Delivery of the fullerene species to either the cytoplasm or nucleus of the cell has been demonstrated. The hydrophobic nature of the fullerene assisting peptide transport is suggested by the effect of gamma-cyclodextrin in lowering the efficacy of transport. These data suggest that the incorporation of a fullerene-based amino acid provides a route for the intracellular delivery of peptides and as a consequence the creation of a new class of cell-penetrating peptides. The peptides were found effective at penetrating the defenses of both liver cancer cells and neuroblastoma cells.

Gold Nanoparticles as Drug Carriers

Gold nanoparticles (AuNPs), in addition to their applications in molecular diagnostics, can be conjugated with peptides, drugs, and other molecules for drug delivery as well as for thermal treatment of cancer. AuNPs have received considerable attention as model drug delivery platforms because of their surface characteristics that enable easy functionalization with chemicals and biological molecules and also due

to their apparently low toxicity (Papasani et al. 2012). Efforts are being made to develop intelligent delivery systems by lining the walls of polymer "delivery-vehicle" particles with gold nanoparticles. By simply shining a laser on loaded delivery vehicles (i.e., particles filled with various contents, such as an enzyme or drug), the walls could be opened and the contents released. This technique has been used successfully for the release of an encapsulated enzyme on demand with a single nanosecond laser pulse. In contrast to the common approach for drug release by changes in the local environment at the site where drug delivery is needed, gold nanoparticle technology enables externally controlled drug release. In addition to drugs, these gold-coated vehicles could be used for the controlled delivery of a wide range of other substances including genes. There is no risk that the laser energy will be significantly absorbed by biological structures such as bodily organs because the absorption of the gold-coated delivery vehicles in the NIR region is intentionally engineered in the wavelength regime for which light has a maximum penetration depth in tissue.

Layered Double Hydroxide Nanoparticles

Layered double hydroxides (LDHs) were well known as catalyst and ceramic precursors, traps for anionic pollutants, catalysts, and additives for polymers. Their successful synthesis on the nanometer scale opened up a whole new application for delivery of drugs and other therapeutic/bioactive molecules (e.g., peptides, proteins, nucleic acids) to mammalian cells. LDH nanoparticles have advantages as well as disadvantages as carriers for nucleic acids and drugs, and some challenges need to be overcome before LDH nanoparticles can be used in a clinical setting (Ladewig et al. 2009). Size-dependent toxicity of LDH was examined in cultured human lung cells; 50-nm particles were determined to be more toxic than larger particles, while LDHs within the size range of 100–200 nm exhibited very low cytotoxicity in terms of cell proliferation, membrane damage, and inflammation response (Choi et al. 2008).

Nanocomposite Membranes for Magnetically Triggered Drug Delivery

Nanocomposite membranes based on thermosensitive, poly(N-isopropylacrylamide)-based nanogels and magnetite nanoparticles have been designed to achieve "on-demand" drug delivery upon the application of an oscillating magnetic field (Hoare et al. 2009). On–off release of sodium fluorescein over multiple magnetic cycles has been successfully demonstrated using prototype membrane-based devices. The total drug dose delivered was directly proportional to the duration of the "on" pulse. The membranes were noncytotoxic, were biocompatible, and retained their switchable flux properties after 45 days of subcutaneous implantation.

Nanocrystals

Nanocrystalline Silver

Silver has been valued for centuries for its medicinal properties. From ancient Greece to the American settlers, silver was used as a preservative for drinking water and other liquid storage. Decades ago, doctors would apply a thin layer of silver to large wounds to prevent infection and promote healing. Nucryst's silver nanocrystalline technology decreases the particle size, thus changing the physical and chemical properties. As the proportion of atoms on the surface increases, the result is a more powerful compound than conventional silver treatments. In vitro tests have demonstrated that active silver clusters of ions begin providing antimicrobial activity immediately and kill many organisms in 30 min, faster than other forms of silver.

Silcryst™ nanocrystals release sustained, uniform doses of silver. Silver nanocrystalline technology is capable of delivering a sustained release of active silver to the dressings over a longer period of time than any other silver treatment. Other treatments, such as silver sulfadiazine and silver nitrate, are characterized by the rapid depletion of active silver, forcing the regular scraping of creams from or applications of solutions to open wounds multiple times per day. This process is labor intensive and extremely traumatic for patients. Silver nanocrystalline technology dressings cover the wound providing sustained release of silver to the dressing, acting as a barrier to infection for up to 7 days. Acticoat™ (Smith & Nephew) dressings for burns and chronic wounds use Nucryst's proprietary Silcryst™ silver nanocrystalline technology. In vitro studies of Acticoat have demonstrated:

- Extensive antimicrobial spectrum of 150 different pathogens
- Rapid kill rates
- Effective against drug-resistant forms of bacteria, such as MRSA (methicillin-resistant *Staphylococcus aureus*) and VRE (vancomycin-resistant Enterococci), sometimes referred to as "superbugs"
- Fast-acting release of ionic silver to the dressing over a sustained period of time (effective for up to 7 days)

The company also is conducting preclinical studies on the use of nanocrystalline silver inhaled into the lungs for the treatment of serious lung infection or lung inflammation. In the future, the company plans to conduct research on the nanocrystalline structures of other metals, including gold, which is well known as a treatment for arthritis, and platinum, which is a well-known treatment for cancer, to determine if the behavior and performance of these metals also can be enhanced.

Elan's NanoCrystal Technology

NanoCrystal® (Elan) particles are small particles of drug substance, typically less than 1,000 nm in diameter, which are produced by milling the drug substance using a proprietary milling technique. The NanoCrystal® particles of the drug

are stabilized against agglomeration by surface adsorption of selected GRAS (generally regarded as safe) stabilizers. The end result is a suspension of the drug substance that behaves like a solution – a NanoCrystal® colloidal dispersion, which can be processed into dosage forms for all routes of administration. NanoCrystal® technology is being used by Johnson & Johnson Pharmaceutical Research & Development in a phase III clinical trial of a long-acting injectable formulation of its paliperidone palmitate in patients with schizophrenia.

NanoCrystal® technology represents a valuable, enabling technology to evaluate new chemical entities which exhibit poor water solubility and is also a valuable tool for optimizing the performance of established drugs. NanoCrystal technology has the potential to rescue a significant number of poorly soluble chemical compounds. The drug in nanoform can be incorporated into common dosage forms, including tablets, capsules, inhalation devices, and sterile forms for injection, with the potential for substantial improvements to clinical performance. There are currently two pharmaceutical products that have been commercialized incorporating NanoCrystal technology, with several additional product launches anticipated over the near future. Advantages of this technology are:

- More rapid absorption of active drug substance
- Higher dose loading with smaller dose volume
- Aqueous based with no organic solvents needed
- Capability for sterile filtering
- Longer dose retention in blood and tumors for some compounds

Biorise System

Biorise system (Aptalis Pharmaceutical Technologies) creates new physical entities by physically breaking down a drug's crystal lattice. This results in drug nanocrystals and/or amorphous drug, which are then stabilized with biologically inert carriers. The carriers used in the Biorise system are biocompatible and readily disperse in the body's GI fluids. The final product is a free-flowing powder that can be incorporated into a variety of dosage forms to achieve the most effective delivery.

Aptalis uses three types of carriers: swellable microparticles, composite swellable microparticles, or cyclodextrins. When used in the Biorise system, all three carrier types improve both solubility and dissolution rates as well as the rate and overall percentage of drug absorption. The selection of the appropriate type of Biorise carrier is a critical step in the process and is dependent upon the drug delivery objective, drug carrier compatibility, and its drug loading capacity.

Aptalis has developed a number of activation systems that can convert a drug into its thermodynamically activated state. These systems provide flexibility and allow the technology to be applied to a range of compounds with differing characteristics. These systems include:

High Energy Mechanochemical Activation (HEMA). This system involves the application of friction and impact energy to the drug, thereby increasing its

entropy and transforming the drug into its activated state. This system is a dry system and maintains the drug/carrier matrix in a powder form at all times.

Solvent-Induced Activation (SIA). This system is particularly suitable for thermolabile compounds and compounds with a low melting point. With this system, a drug can be solubilized in an appropriate solvent and layered onto swellable, cross-linked carriers. Controlled evaporation of the solvent and drying the material create nanoparticles and/or amorphous drug that is stabilized in a carrier.

Super Critical Fluid Activation (SCFA). A drug and carrier are placed in a solvent system within a soluble environment. The solvent is removed by controlled displacement using super critical fluids resulting in the precipitation of nanocrystalline and/or amorphous drug that is stabilized in a carrier.

Before Aptalis begins working on a compound, its experienced teams of scientists evaluate the compound and apply a mathematical model to predict the impact that Biorise will have on a drug. This model simulates an in vitro release profile and also determines the most appropriate carrier system as well as drug to carrier ratios. Modeling is a key component in the Biorise process as it helps to:

- Expedite development programs and accelerate the time to market
- Reduce the need for experimentation
- Speed up the rational screening process
- Rapidly predict the outcome of the project

Aptalis' Biorise system can be used to improve a product already on the market, a drug currently in development, as well as to rescue a drug that has been shelved due to solubility difficulties. The system also offers faster and more efficient processing times compared to other marketed technologies and is currently one of the few bioavailability enhancement technologies that is commercialized and being used in a marketed product. The Biorise system offers additional advantages including:

- No use of surfactants
- Produces a drug powder which can be incorporated into a variety of dosage forms including tablets and capsules
- Stable
- Cost-effective process
- Scaled-up, validated, approved by a regulatory agency and commercialized
- Ability to control and vary the ratio of nanocrystal and amorphous drug
- Uses GRAS materials

Nanodiamonds

Nanodiamonds are nanoparticles varying in size from 2 to 8 nm and can be used for diagnostic as well as therapeutic purposes. Water dispersion of previously insoluble

drugs when complexed with nanodiamonds demonstrates great promise in expanding current drug delivery options (Lam and Ho 2009). Bovine insulin was noncovalently bound to detonated nanodiamonds via physical adsorption in an aqueous solution and demonstrated pH-dependent desorption in alkaline environments of sodium hydroxide (Shimkunas et al. 2009).

Carbon nanodiamonds are much more biocompatible than most other carbon nanomaterials, including carbon blacks, fullerenes, and carbon nanotubes. The noncytotoxic nature of nanodiamonds, together with their unique strong and stable photoluminescence, tiny size, large specific surface area and ease with which they can be functionalized with biomolecules, makes nanodiamonds attractive for various biomedical applications both in vitro and in vivo (Xing and Dai 2009).

Polymer Nanoparticles

Biodegradable polymer nanoparticles include chitosan (CS) nanoparticles, poly(ethylene glycol) (PEG) nanoparticles, and polylactide-co-glycolic acid (PLGA) nanoparticles.

Biodegradable PEG Nanoparticles for Penetrating the Mucus Barrier

Protective mucus coatings typically trap and rapidly remove foreign particles from the eyes, gastrointestinal tract, airways, nasopharynx, and female reproductive tract, thereby strongly limiting opportunities for controlled drug delivery at mucosal surfaces. A preparation of nanoparticles composed of a biodegradable diblock copolymer of polysebacic acid and polyethylene glycol (PSA-PEG), both of which are routinely used in humans, can diffuse through mucous membranes (Tang et al. 2010). In fresh undiluted human cervicovaginal mucus (CVM), which has a bulk viscosity approximately 1,800-fold higher than water at low shear, PSA-PEG nanoparticles diffused at an average speed only 12-fold lower than the same particles in pure water. In contrast, similarly sized biodegradable nanoparticles composed of PSA or PLGA diffused at least 3,300-fold slower in CVM than in water. PSA-PEG particles also rapidly penetrated sputum expectorated from the lungs of patients with cystic fibrosis, a disease characterized by hyperviscoelastic mucus secretions. Rapid nanoparticle transport in mucus is made possible by the efficient partitioning of PEG to the particle surface during formulation. Biodegradable polymeric nanoparticles capable of overcoming human mucus barriers and providing sustained drug release open significant opportunities for improved drug and gene delivery at mucosal surfaces. Beyond their potential applications for cystic fibrosis patients, the nanoparticles also could be used to help treat disorders such as lung and cervical cancer and inflammation of the sinuses, eyes, lungs, and gastrointestinal tract. Chemotherapy is typically given to the whole body and has many undesired side effects. If drugs are encapsulated in these nanoparticles and inhaled directly into the lungs of lung cancer patients, drugs may reach lung tumors more effectively, and

improved outcomes may be achieved, especially for patients diagnosed with early stage NSCLC. PEG acts as a shield to protect the particles from interacting with proteins in mucus that would cause them to be cleared before releasing their contents. Nanoparticles can efficiently encapsulate several chemotherapeutics, and a single dose of drug-loaded particles is able to limit tumor growth in a mouse model of lung cancer for up to 20 days.

Additionally, PEG coating improves the stability of PLGA nanoparticles in the gastrointestinal fluids and helps the transport of the encapsulated protein, tetanus toxoid, across the intestinal and nasal mucous membranes. Furthermore, intranasal administration of these nanoparticles provided high and long-lasting immune responses.

PLGA-Based Nanodelivery Technologies

Polylactide-co-glycolic acid (PLGA) is a FDA-approved copolymer which is used in a host of therapeutic devices, owing to its biodegradability and biocompatibility. PLGA is synthesized by means of random ring-opening copolymerization of two different monomers. PLGA nanoparticles deliver molecules considered too large and complex to transport with known vectors. PLGA is nontoxic, does not illicit an immune response, causes comprehensive transfection, crosses the blood–brain barrier, and supports sustained drug release. PLGA has been successful as a biodegradable polymer because it undergoes hydrolysis in the body to produce the original monomers, lactic acid and glycolic acid. These two monomers under normal physiological conditions are by-products of various metabolic pathways in the body. Since the body effectively deals with the two monomers, there is very minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications. Also, the possibility to tailor the polymer degradation time by altering the ratio of the monomers used during synthesis has made PLGA a common choice in the production of a variety of biomedical devices such as grafts, sutures, implants, and prosthetic devices. As an example, a commercially available drug delivery device using PLGA is Abbott's Lupron Depot® (leuprolide acetate) for the treatment of advanced prostate cancer.

Polymeric Micelles

Micelles are biocompatible nanoparticles varying in size from 50 to 200 nm in which poorly soluble drugs can be encapsulated, which represents a possible solution to the delivery problems associated with such compounds, and could be exploited to target the drugs to particular sites in the body, potentially alleviating toxicity problems. pH-sensitive drug delivery systems can be engineered to release their contents or change their physicochemical properties in response to variations in the acidity of the surroundings. One example of this is the preparation and characterization of novel polymeric micelles (PM) composed of amphiphilic pH-responsive poly(N-isopropylacrylamide) (PNIPAM) or poly(alkyl(meth)acrylate) derivatives. On one hand, acidification of the PNIPAM copolymers induces a coil-to-globule transition

that can be exploited to destabilize the intracellular vesicle membranes. PNIPAM-based PMs, loaded with either doxorubicin or aluminum chloride phthalocyanine, are cytotoxic in murine tumor models. On the other hand, poly(alkyl(meth) acrylate) copolymers can be designed to interact with either hydrophobic drugs or polyions and release their cargo upon an increase in pH. The self-assembly of well-defined polypeptide-based diblock copolymers into micelles and stimuli-responsive behavior of polypeptides to pH and ionic strength is used to produce nanoparticles with controlled size and shape, which are particularly useful for encapsulation and delivery purpose at a controlled pH (Checot et al. 2007).

Chitosan Nanoparticles

Chitin, a polymer, is commercially extracted from shrimp shells and has several medical applications. Chitin is a very large sugar molecule with a large number of acetic acid molecules attached to it. Treatments with soda remove some of this acetic acid from the sugar backbone, converting chitin into biopolymer chitosan. Chitosan is prone to chemical and physical modifications and is very responsive to environmental stimuli such as temperature and pH. These features make chitosan a smart material with great potential for developing multifunctional nanocarrier systems to deliver large varieties of therapeutic agents administrated in multiple ways with reduced side effects. Chitosan modification with a variety of ligands specific for cell surface receptors can increase recognition and uptake of nanocarriers into cells through receptor-mediated endocytosis (Duceppe and Tabrizian 2010).

Chitosan nanoparticles are known for their ability to overcome biological barriers and facilitate the delivery of complex drugs such as insulin, vaccines, plasmid DNA, and genes. In the NanoBioSaccharides project which is financed by the European Union, scientists from universities and commercial companies in Germany, France, Spain, Denmark, and Italy will collaborate to optimize these technologies to further improve the delivery of macromolecules, e.g., insulin, via the nasal, pulmonary, and oral routes instead of via an injection into the blood vessels.

N-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC) is water-soluble derivative of chitosan (CS), synthesized by the reaction between glycidyl-trimethyl-ammonium chloride and CS. HTCC nanoparticles have been formed based on ionic gelation process of HTCC and sodium tripolyphosphate (TPP). Bovine serum albumin (BSA), as a model protein drug, is incorporated into the HTCC nanoparticles measuring 110–180 nm in size with encapsulation efficiency up to 90 %. In vitro release studies showed a burst effect, and a slow and continuous release followed. Encapsulation efficiency was obviously increased with increase of initial BSA concentration.

Coating of PLGA nanoparticles with the mucoadhesive CS improves the stability of the particles in the presence of lysozyme and enhanced the nasal transport of the encapsulated tetanus toxoid. Nanoparticles made solely of CS are stable upon incubation with lysozyme. Moreover, these particles are very efficient in improving the nasal absorption of insulin as well as the local and systemic immune responses to tetanus toxoid, following intranasal administration.

By encapsulating drugs in CS cubes, the cells of the body are tricked into absorbing drugs that could not normally be transported across the cell membrane. After delivering its load directly into the cells affected, CS is broken down in the body and disappears without a trace. CS capsules can transport siRNA into the cell to switch off faulty genes selectively. A siRNA against the respiratory syncytial virus (RSV), RSV-NS1gene (siNS1), has been tested for its potential in decreasing RSV infection and infection-associated inflammation in rats (Kong et al. 2007). Plasmids encoding siNS1 were complexed with a chitosan nanoparticle delivery agent and administered intranasally. They were shown to be effective in reducing virus titers in the lung and in preventing the inflammation and airway hyperresponsiveness associated with the infection.

QDs for Drug Delivery

Engineered QDs with integrated targeting, imaging, and therapeutic functions are excellent materials for study of drug delivery in cells and experimental animals. They can provide important information for the rational design of biocompatible drug carriers and can serve as a superior alternative to magnetic and radioactive imaging contrast agents in preclinical drug screening, validation, and delivery research (Qi and Gao 2008). In a multifunctional QD coated with amphiphilic polymer, hydrophobic drugs can be trapped between the nanoparticle core and amphiphilic polymer coating layer, and hydrophilic targeting molecules can be immobilized on the outer surface.

To meet some challenges of drug delivery, the engineered QDs would be able to stabilize therapeutic compounds, increase their plasma circulation time while reducing the concentration of free drug to minimize unwanted side effects, and to release the drug with a well-controlled profile. In addition, it should be possible to covalently link the therapeutic compounds to the target on QD surface via cleavable chemical bonds, so that the bioconjugates are initially large enough to avoid renal filtration but, following cleavage of the ligands, are small enough to be cleared out of the body. With advances being made in the identification of new targeting ligands, the development of specialized nanoparticles and the discovery of better conjugation techniques, the QD-based drug delivery has excellent prospects. Role of QDs in drug delivery for cancer is described later in this chapter.

Special Procedures in Nanoparticle-Based Drug Delivery

Coated Nanoparticles for Penetrating Cell Membranes Without Damage

Most nanoscale objects are typically internalized by cells into membrane-bounded endosomes and fail to access the cytosolic cell machinery. A study has shown that gold nanoparticles coated with alternating bands of two different types of molecules can penetrate a cell quickly, passing into cells without damaging them, while those

randomly coated with the same materials cannot (Verma et al. 2008). This is the first fully synthetic material that can pass through a cell membrane without rupturing it and is significant for drug delivery across biological membranes. In addition to the practical applications of such nanoparticles for drug delivery, they have been used to deliver fluorescent imaging agents to cells that could help explain how some biological materials such as peptides are able to enter cells.

Combinatorial Synthesis of Nanoparticles for Intracellular Delivery

Evaluation of a large library of structurally distinct nanoparticles with cationic cores and variable shells was carried out by using robotic automation. Nanoparticles were combinatorially cross-linked with a diverse library of amines, followed by measurement of molecular weight, diameter, RNA complexation, cellular internalization, and in vitro siRNA and pDNA delivery (Siegwart et al. 2011). Analysis revealed structure–function relationships and beneficial design guidelines. Cross-linkers optimally possessed tertiary dimethylamine or piperazine groups and potential buffering capacity. Covalent cholesterol attachment enabled intracellular delivery in vivo to liver hepatocytes in mice.

Drug Delivery Using "Particle Replication in Nonwetting Templates"

Most current techniques for particle formation are incompatible with organic materials because they involved baking, etching, or processing robust metals using with solvents that destroy fragile organic matter such as genes or drugs. The relatively simple process, called Particle Replication in Nonwetting Templates (PRINT), avoids creating films or "scum layers" that would clump particles together rather than allowing them to be harvested independent of one another. PRINT affords the simple, straightforward encapsulation of a variety of important bioactive agents, including proteins, DNA, and small-molecule therapeutics, which indicates that PRINT can be used to fabricate next-generation particulate drug delivery agents. The particles are so small they can be designed and constructed to measure <200 nm in diameter. The method avoids harsh treatment but also allows formation of uniform particles in any shape that designers choose: spheres, rods, cones, trapezoidal solids, etc. Besides drug delivery, this technology will have a profound impact on human health care in areas such as chemotherapy, gene therapy, and disease detection. Particles injected into the body can be designed to be biodegradable and incorporate as "cargo" any biological material that designers want to introduce into patients' bloodstreams for more efficient uptake by cells for diagnostic testing or therapy. Preliminary in vitro and in vivo studies have demonstrated future utility of PRINT particles as delivery vectors in nanomedicine (Gratton et al. 2007).

Encapsulating Water-Insoluble Drugs in Nanoparticles

Many of the most potent anticancer agents are poorly soluble in water, presenting a challenge for medicinal chemists who must develop methods of delivering these

drugs in the watery environment of the human body. Nanoparticles appear to be perfectly suited to this task, and indeed, numerous research groups are developing nanoparticles specifically for delivering water-insoluble drugs to tumors. A fundamental understanding of particle size control in antisolvent precipitation is beneficial for designing mixing systems and surfactant stabilizers for forming nanoparticles of poorly water-soluble drugs with the potential for high dissolution rates.

Inverse emulsion photopolymerization is a method that uses light to create a well-defined polymeric nanoparticle with internal spaces that can provide a friendly environment to water-insoluble drugs and channels through which the entrapped drugs can escape into malignant cells (Missirlis et al. 2006). The investigators created these nanoparticles from two different polymers that cross-link to each other when exposed to light from an argon laser for 1 h. They then added the nanoparticles to a solution of doxorubicin and evaporated the solvent used to dissolve the anticancer drug. Nearly half of the drug in solution became encapsulated within the nanoparticles. The researchers note that the resulting nanoparticles contain a protein-repelling surface coating that should result in favorable pharmacokinetic behavior. Experiments to test the drug release characteristics of these nanoparticles showed that maximum release occurred at approximately 8 h and then remained close to that level for a week. The data imply that release occurs through a diffusion mechanism, i.e., drug travels through channels in the nanoparticle to the nanoparticle surface, as opposed to a disintegration mechanism in which the nanoparticle falls apart and releases drug. This novel colloidal system can be as a controlled delivery system for small hydrophobic drugs for cancer.

Filomicelles Versus Spherical Nanoparticles for Drug Delivery

Shape may be important in designing better nanotechnology-based drug delivery vehicles. A study in rodents has compared highly stable, polymer micelle assemblies known as filomicelles with spheres of similar chemistry and shown that filomicelles persisted in the circulation up to 1 week after intravenous injection (Geng et al. 2007). This is about ten times longer than their spherical counterparts and is more persistent than any known synthetic nanoparticle. Under fluid flow conditions, spheres and short filomicelles are taken up by cells more readily than longer filaments because the latter are extended by the flow. Preliminary results further demonstrate that filomicelles can effectively deliver the anticancer drug paclitaxel and shrink human-derived tumors in mice. Although these findings show that long-circulating vehicles need not be nanospheres, they also lend insight into possible shape effects of natural filamentous viruses.

Flash NanoPrecipitation

Flash NanoPrecipitation produces stable nanoparticles at high concentrations using amphiphilic diblock copolymers to direct self-assembly (Prudhomme et al. 2006). In NanoPrecipitation, two streams of liquid are directed toward one another in a confined area. The first stream consists of an organic solvent that contains the medicines and imaging agents, as well as long-chain molecules called polymers.

The second stream of liquid contains pure water. When the streams collide, the hydrophobic medicines, metal imaging agents, and polymers precipitate out of solution in an attempt to avoid the water molecules. The technique has been applied to the anticancer agent paclitaxel. The polymers immediately self-assemble onto the drug and imaging agent cluster to form a coating with the hydrophobic portion attached to the nanoparticle core and the hydrophilic portion stretching out into the water. By carefully adjusting the concentrations of the substances, as well as the mixing speed, the researchers are able to control the sizes of the nanoparticles. Uniform particles with tunable sizes from 50 to 500 nm can be prepared. The key to the process is the control of time scales for micromixing, polymer self-assembly, and particle nucleation and growth. The diffusion-limited assembly enables particles of complex composition to be formed. The stretched hydrophilic polymer layer keeps the particles from clumping together and prevents recognition by the immune system so that the particles can circulate through the bloodstream. The hydrophobic interior of the particles ensures that they are not immediately degraded by watery environments, though water molecules will, over time, break the particles apart, dispersing the medicine. Ideally, the particles would persist for 6–16 h after they are administered intravenously, which would allow enough time for the potent packages to slip into the solid tumor cells whenever they encounter them throughout the body.

Applications include controlled delivery of multiple drugs from nanoparticles as well as aerosol drug delivery. It enables the simultaneous encapsulation and controlled release of both hydrophobic and hydrophilic actives. The incorporation of gold nanoparticles and organic compounds into single nanoparticles enables simultaneous delivery and medical imaging. Finally, the ability to dry the nanoparticles by lyophilization or spray drying and to reconstitute them without aggregation greatly enhances the applicability of the technology.

These nanoparticles can deliver medicine deep into the lungs or infiltrate cancer cells while leaving normal ones alone. Only 100–300 nm wide, the particles can be loaded with medicines or imaging agents, like gold and magnetite, that will enhance the detection capabilities of CT scans and MRIs. The nanoparticles are too large to pass through the membrane of normal cells but will pass through larger defects in the capillaries in rapidly growing solid tumors.

Particles in this size range also could improve the delivery of inhaled drugs because they are large enough to remain in the lungs, but too small to trigger the body's lung-clearing defense systems. This trait could maximize the effectiveness of inhaled, needle-free vaccination systems. It has potential applications in the development of nanoparticle-based aerosol vaccines for tuberculosis and diphtheria. Because of their potential for use on a large scale at relatively low cost, these systems are particularly attractive for use in the developing world.

Magnetic Nanoparticles for Drug Delivery

These particles have a magnetic core with a polymer or metal coating, which can be functionalized. A therapeutic agent such as a cytotoxic drug or a therapeutic DNA can be attached to the particle. The particles are delivered by a catheter close

to the site of action and further guided by external magnetics. Investigations of magnetic micro- and nanoparticles for targeted drug delivery began over 30 years ago, and major advances have been made in particle design and synthesis techniques, which have been reviewed (McBain et al. 2008). Although very few clinical trials have taken place, with this technique, it appears to be promising. nanoTherics is developing magnefect-nano™, which has the following advantages:

- Up to 1,000-fold higher transfection efficiencies at short transfection times when compared to cationic lipid reagents
- No adverse effects on cell viability
- Potential to target/penetrate physical barriers in vivo (e.g., mucous layers for cystic fibrosis gene transfection)
- Successful with "hard to transfect" cells/cell lines
- Cost-effective, saving time and materials
- Can be used with adherent and suspension cells
- Scalable for high-throughput screening

Nanoparticles Bound Together in Spherical Shapes

Altair Nanotechnologies Inc has developed unique micron-size structures (TiNano Spheres™) made by its patented "growth-in-film" nanotechnology. They consist of hundreds of nanoparticles bound together in spherical and near spherical shapes and are capable of carrying active pharmaceutical ingredients (API), biocides, or fungicides on either the interior or exterior surfaces. The nanoparticles have a very high surface area and when coated with an API delivers a very large amount of drug to biosystem interface. This larger interface could improve solubility and/or reaction rates.

Altair's nanotechnology is used to create competent porous microstructures consisting of high surface area nano-primary particles to enable new applications for hard to dissolve drugs. A sustained release of drugs is possible by applying the drug to the inside of the TiNano Spheres™. Dual action properties are possible by applying one drug to the inside and another to the outside of the TiNano Sphere. Altair has successfully deposited at least one of these drugs on the surface of TiNano Spheres™. Some of the many possible applications of TiNano Spheres™ are:

- Drug delivery by topical applications
- Sustained release of antibiotic and fungicides
- Sustained release of drugs for cholesterol lowering
- Pain and itch preparations with sustained-release action
- Sunscreen and after-sun care

Perfluorocarbon Nanoparticles for Imaging and Targeted Drug Delivery

Perfluorocarbon (PFC) nanoparticles are approximately 200 nm in diameter and are encapsulated in a phospholipid shell, which provides an ideal surface for the

incorporation of targeting ligands, imaging agents, and drugs. PFC nanoparticles can serve as a platform technology for molecular imaging and targeted drug delivery applications. For molecular imaging, PFC nanoparticles can carry very large payloads of gadolinium to detect pathological biomarkers with MRI. A variety of different epitopes, including $\alpha v\beta 3$, tissue factor, and fibrin, have been imaged using nanoparticles formulated with appropriate antibodies or peptidomimetics as targeting ligands. Lipophilic drugs can also be incorporated into the outer lipid shell of nanoparticles for targeted delivery. Upon binding to the target cell, the drug is exchanged from the particle surfactant monolayer to the cell membrane through a novel process called "contact-facilitated drug delivery." By combining targeted molecular imaging and localized drug delivery, PFC nanoparticles provide diagnosis and therapy with a single agent and would facilitate the development of personalized medicine (Winter et al. 2007).

The contrast agents in development by Kereos Inc comprise tiny perfluorocarbon nanoparticles suspended in an emulsion. Agents such as technetium-99 m may be attached to the nanoparticles to provide the contrast that allows for imaging. In addition, nanoparticles are labeled with a specific ligand that causes the agent to target newly developing blood vessels. When injected into the body, the resulting agent will find and illuminate these vessels. Anticancer drugs and therapeutic radionuclides may also be incorporated into the nanoparticles to deliver therapy directly and selectively.

Prolonging Circulation of Nanoparticles by Attachment to RBCs

Polymeric nanoparticles are used as carriers for systemic and targeted drug delivery. They protect drugs from degradation until they reach their target and provide sustained release of drugs. However, applications of nanoparticles are limited by their short in vivo circulation lifetimes. They are quickly removed from the blood, sometimes in minutes, rendering them ineffective in delivering drugs. It is now possible to dramatically improve the in vivo circulation lifetime of polymeric nanoparticles by attaching them to the surface of red blood cells (RBCs) without affected their circulation (Chambers and Mitragotri 2007). The particles remain in circulation as long as they remain attached to RBCs, theoretically up to the circulation lifetime of a RBC, which is 120 days. Particles eventually detach from RBCs due to shear forces and cell–cell interactions and are subsequently cleared in the liver and spleen.

The researchers have learned that particles adhered to RBCs can escape phagocytosis because red blood cells have a knack for evading macrophages. Nanoparticles are not the first to be piggybacking on red blood cells; the strategy has already been adopted by certain bacteria, such as *Hemobartonella*, that adhere to RBCs and can remain in circulation for several weeks. Using RBCs to extend the circulation time of the particles avoids the need to modify the surface chemistry of the entire particle, which offers the potential to attach chemicals to the exposed surface for targeting applications. The exposed surface of the particles could be used to immobilize enzymes and improve their in vivo circulation lifetime. The enzyme would have direct access to plasma in the systemic circulation. RBC-mediated

prolonged circulation may also be applied to gene delivery applications in which extended circulation times are difficult to achieve. Synthetic gene delivery vectors suffer from rapid clearance by the reticuloendothelial system, restricting transfection to the liver and lung. RBC attachment of gene vectors may provide a long-circulating depot, thereby increasing their residence time in blood.

RBC membrane-coated nanoparticles present a major breakthrough in drug delivery technology and show great promise for clinical applications (Fang et al. 2012). This technique could be applied for the delivery of drugs and circulating bioreactors in a wide variety of conditions such as cancer and heart disease.

Self-Assembling Nanoparticles for Intracellular Drug Delivery

EAK16-II, a self-assembling peptide, has been found to stabilize the hydrophobic anticancer agent ellipticine (EPT) in aqueous solution and form nanoparticles with an average size of ~100 nm (Bawa et al. 2011). This nanoformulation is cytotoxic to human lung carcinoma A549 cells that is comparable to EPT dissolved in dimethyl sulfoxide. It enhances EPT uptake significantly as compared to the microformulation. Promising therapeutic efficacy, specific delivery pathway, and intracellular distribution pattern discovered in this study may help further develop EPT as a nanoformulation for clinical applications.

Researchers at the University of Ulsan College of Medicine (Seoul, Korea) have developed self-assembling nanoparticles that can sense the low pH of endosomes and disintegrate, which not only releases their drug payload but enables it to exit the endosomes. Chitosan serves as the starting material for these self-assembling nanoparticles. The investigators modify the polymer by attaching a chemical derivative of the amino acid histidine to each of the sugar units in the chitosan backbone. At neutral pH, histidine is hydrophobic, or poorly soluble in water. The presence of multiple histidines on the water-soluble, or hydrophilic, chitosan backbone creates a molecule that naturally self-assembles into a structure that surrounds the hydrophobic histidines with a protective shell of hydrophilic chitosan. When added to cells grown in culture, the nanoparticles fuse with the cell membrane, forming endosomes inside the cell. At the low pH found inside an endosome, histidine takes on a positive charge and also becomes hydrophilic. As a result, the physical forces that held together the self-assembling nanoparticle no longer exist, and the nanoparticle falls apart. Any drug molecules entrapped within the nanoparticle is then released into the endosomes.

Trojan Nanoparticles

Trojan particles combine the drug release and delivery potential of nanoparticle systems with the ease of flow, processing, and aerosolization potential of large porous particle systems by spray drying solutions of polymeric and nonpolymeric nanoparticles into extremely thin-walled macroscale structures. These hybrid particles exhibit much better flow and aerosolization properties than the nanoparticles; yet, unlike the large porous particles, which dissolve in physiological conditions to

produce molecular constituents, hybrid particles dissolve to produce nanoparticles with drug release and delivery. Formation of the large porous nanoparticle aggregates occurs via a spray-drying process that ensures the drying time of the sprayed droplet is sufficiently shorter than the characteristic time for redistribution of nanoparticles by diffusion within the drying droplet. Additional control over the physical characteristics is achieved by adding other components to the spray-dried solutions, including sugars, lipids, polymers, and proteins. The ability to produce large porous nanoparticles appears to be largely independent of molecular component type as well as the size or chemical nature of the nanoparticles.

These particles range in size from 25 nm to several hundred nms and can be used to deliver drugs to specific sites within the body. They are robust drug delivery systems that can be used to encapsulate drugs of varying chemistry and molecular weights. Trojan nanoparticles will be discussed further in connection with drug delivery across the BBB.

Therapeutic Protein Delivery from Nanoparticle–Protein Complexes

Scientists at the US Department of Energy's Brookhaven National Laboratory have attached gold nanoparticles to proteins to form sheets of protein–gold arrays (Hu et al. 2007). The nanoparticle–protein complexes can be used to identify functional parts of proteins and to construct new protein complexes, which can be used as precision vehicles for targeted drug delivery.

Therapeutic protein delivery from nanoparticle aggregates is being developed and is based on several independent variables including nanoparticle size and chemical composition of the particle. The nanoparticle aggregate technology allows remarkable versatility in protein loading and subsequent release. Optimization of a formulation can be achieved in a relatively short time for a given protein drug. Importantly, this technology can substantially reduce the "burst release" of the protein, which occurs with other delivery systems. In preclinical animal studies, the ability to control the release of the protein for periods of 3 months and greater has been established. Additional preclinical animal studies have shown that the materials used to produce the aggregates, which are included in several FDA-approved products, are biocompatible and therefore suitable for use in a drug delivery system. It represents one of the simplest delivery systems with great versatility for incorporating and delivering proteins. The abilities to load the protein in a cost-effective manufacturing process without using solvents or polymerization and to tightly control the drug release profile are potentially very significant advantages over other protein delivery technologies. Protein delivery is important as therapeutic proteins are being increasingly used to treat a wide variety of disease including cancer, infections, rheumatoid arthritis, and autoimmune diseases.

Triggered Release of Drugs from Nanoparticles

Controlling location and timing of the release enables use of potent drugs in a personalized manner so that the interaction with the right target is ensured. One method for this is externally triggered release of encapsulated compounds, which can be accomplished if drug delivery vehicles, such as liposomes or polyelectrolyte

multilayer capsules, incorporate NP actuators. NPs can efficiently harvest energy from tissue penetrating, external stimuli, such as near infrared (NIR) light and alternating magnetic fields (AMFs), localize it by local field enhancement or convert it into heat, and thus trigger release of cargo from thermoresponsive vehicles. Cargo release can be externally triggered by magnetic or electromagnetic fields from vesicles loaded with superparamagnetic or metallic NPs. Control over the assembly of NPs into a responsive vesicle determines the vesicle stability, and the ability to control timing and dose of the cargo released from the vesicle. Thus, nondestructive, reversible changes in permeability of delivery vehicles enable pulsed cargo release, and therefore a close control over timing and dose of released cargo.

Liposomes

Basics of Liposomes

Liposome properties vary substantially with lipid composition, size, surface charge, and the method of preparation. They are therefore divided into three classes based on their size and number of bilayers:

1. Small unilamellar vesicles are surrounded by a single lipid layer and measure 25–50 nm in diameter.
2. Large unilamellar vesicles are a heterogeneous group of vesicles similar to and are surrounded by a single lipid layer.
3. Multilamellar vesicles consist of several lipid layers separated from each other by a layer of aqueous solution.

Lipid bilayers of liposomes are similar in structure to those found in living cell membranes and can carry lipophilic substances such as drugs within these layers in the same way as cell membranes. AFM is useful in evaluating the physical characteristics and stability of liposomes as drug delivery systems (Spyratou et al. 2009). The pharmaceutical properties of the liposomes depend on the composition of the lipid bilayer and its permeability and fluidity. Cholesterol, an important constituent of many cell membranes, is frequently included in liposome formulations because it reduces the permeability and increases the stability of the phospholipid bilayers. Until recently, the use of liposomes as therapeutic vectors was hampered by their toxicity and lack of knowledge about their biochemical behavior. The simplest use of liposomes is as vehicles for drugs and antibodies for the targeted delivery of anticancer agents. Furthermore, liposomes can be conjugated to antibodies or ligands to enhance target-specific drug therapy.

Stabilization of Phospholipid Liposomes Using Nanoparticles

A simple strategy of mixing phospholipid liposomes with charged nanoparticles and using sonication to mix them at low volume fraction produces particle-stabilized

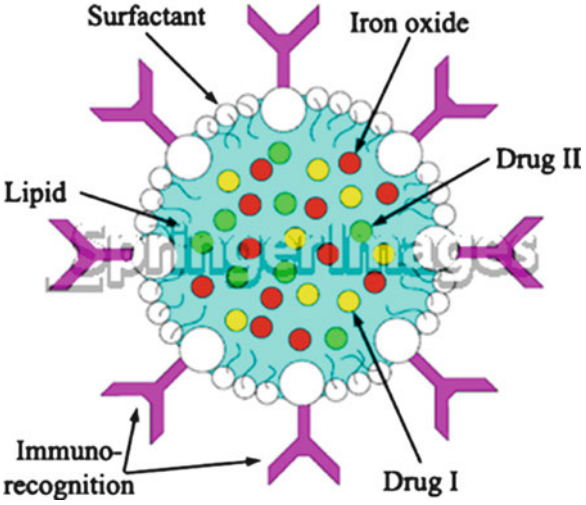

Fig. 5.3 Schematic image of a lipid nanoparticle (© Springer Science+Business Media LLC)

liposomes that repel one another and do not fuse (Zhang and Granick 2006). Subsequently, the volume fraction can be raised as high as 50 %, reversibly, still without fusion. The nanoparticles adhere to the capsules and prevent further growth, freezing them at the desired size. The lipid concentration can then be increased without limits. As proof of concept, fluorescent dyes were encapsulated within lipid capsules. No leakage occurred, and the lipids proved stable against further fusion. Although these particle-stabilized liposomes were stable against fusion, 75 % of the outer liposome surface remained unoccupied.

This opens the door to using particle-stabilized liposomes in various applications. The biocompatible containers could carry cargo such as enzymes, DNA, proteins, and drug molecules throughout living organisms. They could also serve as surrogate factories where enzyme-catalyzed reactions are performed. By attaching biomolecules to the capsule's surface, novel colloidal-size sensors could be produced. An additional use for stabilized lipid capsules is the study of behavior of a drug contained in this nanoenvironment.

Lipid Nanoparticles

Lipid nanoparticles are made up of lipids that assemble on their own into spherical particles or liposomes. This technology differs from conventional liposome technology in that the lipids used contain polymerizable functional groups that are cross-linked when exposed to UV light. As a result, the surface of the polymerized nanoparticle more closely resembles a nanosphere or bead than a droplet of fat (liposome), and drugs/targeting agents can be attached. Schematic image of a lipid nanoparticle is shown in Fig. 5.3.

Advantages of the lipid nanoparticle technology are:
Characteristics of lipid nanoparticles are:

- Unlike conventional bilayer liposomes, they do not randomly fuse with themselves or other membranes.
- The surface character, in terms of charge and molecular makeup, is easily modified.
- The spherical assemblies are easy to synthesize and very stable.
- Multivalent presentation of small-molecule ligands on nanoparticles offers vastly improved performance over the ligand alone.
- In addition to the multivalent attachment of ligands/antibodies to the surface, nanoparticles can carry a high payload of radiation, chemotherapeutic, or imaging agent to the target cell.
- Since the nanoparticle is larger than either antibodies or small-molecule ligands, the complex does not quickly leak from the blood vessel. As a result, the biodistribution and safety profile of the drug can be significantly improved.

Applications of Lipid Nanoparticles

The nanoparticle technology has broad therapeutic and diagnostic applications. The multivalent presentation of ligands or antibodies on nanoparticles makes this new class of drug ideally suited to treat diseases, which involve proliferation of blood vessels such as cancer, atherosclerosis, apoptosis, inflammation, rheumatoid arthritis, macular degeneration, unstable plaque, stroke, heart disease, and psoriasis.

When nanoparticles are used in the treatment of cancer, their powerful targeting ability and potential for large cytotoxic payload dramatically enhance the efficacy of conventional pharmaceuticals as well as novel therapeutics such as gene therapy, radioimmunotherapy, and photodynamic therapy. Integrin-targeted nanoparticles can be used for site-specific delivery of a therapeutic payload by using an anticancer gene. These targeted nanoparticles can deliver radionuclides and chemotherapeutics to tumors. Further applications are discussed under drug delivery for cancer.

Polymerized Liposomal Nanoparticle

Polymerized liposomal nanoparticle (PLN) is a nonviral nanoparticle incorporating a customizable drug delivery system for chemotherapeutic applications. PLNs are created using the self-assembling ability of a unique class of diacetylenic lipids that can be polymerized into stable, bimolecular membrane structures capable of delivering a drug payload. The PLN technology lends itself especially well to the display of multiple functions. These particles are comprised of individual lipid monomers, part of which are functionalized for the purpose of targeting and may include additional moieties to control other physical properties such as surface charge, polarity, and fluidity. Different functionalized lipids can be rapidly mixed and matched in an infinite number of combinations and relative concentrations to create tailor-made particles with desirable targeting and circulation properties. The nanoparticles are

nonimmunogenic, display no acute toxicity, and can be highly concentrated. Intracellular degradation and excretion rates of the particles can be modulated by controlling the degree of polymerization.

Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLNs) have emerged as oral bioavailability enhancer vehicles for various drugs. The protective effect of SLNs, coupled with their sustained/controlled-release properties, prevents drugs/macromolecules from premature degradation and improves their stability in the gastrointestinal tract. Review of various publications reveals that direct oral administration of SLNs improves the bioavailability of drugs 2- to 25-fold (Harde et al. 2011).

Lipid Nanocapsules

Due to their small size, lipid nanocapsules (LNC) might be promising as an injectable as well as for an oral drug delivery system. LNC provides sufficient drug solubility to avoid embolization during intravenous injection and facilitates drug absorption after oral administration. Biocompatible ibuprofen LNC has been developed with a particle size of ~50 nm. Pain relief after intravenous administration of ibuprofen is prolonged by at least 2 h when administering LNC formulation. A drug delivery system for intravenous administration of ibuprofen is available, which exhibits sustained-release properties by either oral or intravenous route and could be useful in the treatment of postoperative pain. LNCs can also be used as a transdermal drug delivery system as described later in this chapter.

Lipid Emulsions with Nanoparticles

An artificial lipoprotein-like particle, lipid nanosphere (LNS), is 25–50 nm in diameter and is composed of soybean oil and egg lecithin. Because of the lower uptake of LNS particles containing dexamethasone palmitate by the liver, LNS shows good recovery from the liver and prolonged the plasma half-life of after intravenous injection. In addition, higher anti-inflammatory efficacy of LNS is observed in targeting of dexamethasone palmitate into sites of inflammation. LNS easily and selectively pass through the leaky capillary wall by passive diffusion depending on the plasma concentration. LNS seems to be a promising carrier system for passive targeting of lipophilic drugs.

LNS has also been studied as a low-dose therapeutic system for amphotericin B (AmB), a potent antifungal drug. As a small-particle lipid emulsion, LNS is

taken up by the liver to a lesser extent than a conventional lipid emulsion. As a result, LNS yields higher plasma concentrations of a radiochemical tracer than does the conventional lipid emulsion. LNS incorporating AmB (LNS-AmB) is a homogeneous emulsion with mean particle diameters ranging from 25 to 50 nm and yields higher plasma concentrations of AmB than Fungizone, a conventional intravenous dosage form of AmB, following intravenous administration in laboratory animals. This difference between LNS-AmB and Fungizone is also observed for constant intravenous infusion. In contrast to Fungizone, LNS-AmB shows a linear relationship between dose and area under the curve (AUC). These pharmacokinetic characteristics of LNS-AmB make it a suitable candidate for an effective low-dose therapeutic system for AmB.

Nanolipispheres are colloidal systems of drugs in a solid lipid matrix. These systems possess a submicron mean diameter and a uniform size distribution. A microemulsion–solidification process for manufacture produces a suspension of solid nanoparticles, which is then dried to obtain physically stable nanolipispheres in a powder form. Nanolipispheres provide for:

- Carrier incorporation of lipophilic and hydrophilic drugs
- Oral delivery of macromolecules that can be absorbed as a whole or as fragments through the gastrointestinal tract
- Therapeutic efficacy of some drugs by preferential and consistent absorption and metabolism through the lymphatic system
- Modified drug release

Nanotechnology has been applied to improve the absorption of CoQ10, a lipid-soluble compound found in the mitochondria of all living cells. It is a powerful antioxidant that is essential in the production of cellular energy and has been clinically shown to support healthy heart function, regulate blood pressure, increase energy and vitality, scavenge free radicals, and enhance the immune system. Although endogenously produced in the liver, there are conditions in which adequate production of CoQ10 in the body is impaired. In such situations, supplementation of CoQ10 has been shown to be very beneficial. However, many currently available dosage forms of CoQ10 exhibit negligible dissolution properties indicating potentially poor bioavailability, thereby limiting the therapeutic effect.

CoQ10-loaded PLGA nanoparticles, produced by a scalable emulsion–diffusion–evaporation method and measuring <100 nm, have been shown to significantly quench reactive oxygen species (ROS) with nearly tenfold higher efficacy than free CoQ10 (Swarnakar et al. 2011). Further, positively charged CoQ10-NPs were localized in two major sources of ROS generation: mitochondria and lysosomes. CoQ10 nanoparticles showed improved oral bioavailability (4.28 times) as compared to free CoQ10. The higher anti-inflammatory activity of CoQ10 nanoparticles is attributed to significant accumulation of these in the inflamed tissues.

Nanostructured Organogels

Organic gel nanomaterials can be used to encapsulate pharmaceutical, food, and cosmetic products. Using olive oil and six other liquid solvents, a simple enzyme has been added to chemically activate a sugar that changes the liquids to organic gels, thus using building blocks provided by nature to create new nanomaterials that are completely reversible and environmentally benign (John et al. 2006). In this study, researchers activated a sugar using a simple enzyme, which generated a compound that self-assembles into 3D fibers measuring approximately 50 nm in diameter. As the fibers entangle, a large amount of solvent gets packed together, trapping some 10,000 molecules.

Limitations of Liposomes for Drug Delivery

The use of liposomes may be limited because of problems related to stability, the inability to deliver to the right site, and the inability to release the drug when it gets to the right site. However, liposome surfaces can be readily modified by attaching polyethylene glycol (PEG) units to the bilayer (producing what is known as stealth liposomes) to enhance their circulation time in the bloodstream.

Several attempts have been made to use liposomes, targeted by specific ligands, for the delivery of antithrombotic/thrombolytic agents in order to increase their efficacy and decrease side effects. Although liposomes loaded with various antithrombotic drugs have been the subject of a significant number of experimental studies, they are not considered as candidates for clinical application in the near future (Elbayoumi and Torchilin 2008).

Liposomes Incorporating Fullerenes

Buckysomes are a new generation of liposomes that incorporate fullerenes to deliver drugs that are not water soluble, or tend to have large molecules, and are very hard to get into the body. Buckysomes appear to have much more flexibility in incorporating a wider range of drugs, as well as large molecule drugs, and delivering and releasing them more effectively. Buckysomes are being investigated for delivery of cancer therapeutics and anesthesia.

A study has examined the antioxidant activity of fullerene-C60 incorporated in liposome with a diameter of 75.6 nm, which was shown to have an antioxidant action characterized by long-term persistence, and is attributed to fullerene-C60 but hardly to liposome in all the tests examined (Kato et al. 2011). The combination is expected to be effective as a skin-protecting agent against oxidative stress.

Arsonoliposomes

Arsonolipids are analogs of phosphonolipids, in which P has been replaced by arsenic (As). Although arsonolipids possess interesting biophysical and biochemical properties, their anticancer or antiparasitic activity is not considered adequate for therapeutic applications. But when arsonolipids are incorporated in liposomes, arsonoliposomes show increased toxicity against cancer cells (compared to that of arsenic trioxide) but at the same time were less toxic than arsenic trioxide for normal cells (Fatouros et al. 2006). Furthermore, arsonoliposomes also demonstrate antiparasitic activity in vitro. Nevertheless, As is rapidly cleared from blood after in vivo administration of arsonoliposomes, and this will highly limit possible therapeutic applications. In addition, the fact that arsonoliposomes were observed to aggregate and subsequently fuse into larger particles in the presence of cations may also be considered as a problem. Thereby, methods to modulate the stability of arsonoliposomes and, perhaps, their in vivo distribution (as surface property modification) are currently being investigated. It has been shown that arsonoliposome pegylation results in the formation of liposomes with very high membrane integrity. In addition, pegylation results in increased physical stability of arsonoliposomes and abolishment of cation-induced aggregation and fusion. Nevertheless, further in vivo studies are required in order to prove if pegylation alters arsonoliposome in vivo kinetics in a positive way, without affecting their activity. Further development of arsonoliposomes to develop therapeutic systems for cancer or parasitic diseases is justified. Toxicity issues would need to be resolved

Liposome–Nanoparticle Hybrids

Small iron nanoparticles, quantum dots, liposomes, silica, and polystyrene nanoparticles have been incorporated into liposomes for a variety of applications. Different techniques to achieve encapsulation of solid or semisolid nanoparticles within liposomes have been described. These offer improvements in nanoparticle aqueous solubilization and offer a viable platform (the liposome surface) for further bioconjugation. Moreover, these hybrids have increased survival time in blood circulation following systemic administration and accumulate at sites of leaky vasculature such as in tumors or inflammatory lesions providing opportunities for a combination of diagnostic imaging and therapeutics (Al-Jamal and Kostarelos 2007). Some examples of liposome–nanoparticle hybrids and their applications are shown in Table 5.3.

Table 5.3 Liposome–nanoparticle hybrid systems

Nanoparticle	Method of encapsulation	Rationale	Applications
Superparamagnetic iron oxide (SPIO) particles/magnetite	Lipid film hydration and sonication	Cationic magnetoliposome provides selective intracellular hyperthermia and immune response induction	Cancer therapy
Quantum dots PEG-coated QD	QDs–liposome electrostatic complexation	High intracellular QD delivery	Intracellular trafficking
Phospholipid vesicles	Glass bead method	Preparation of double liposomes, which retard the drug release	Vaccines Drug delivery
Silicon-based nanoparticles	Adsorption and rupture of small unilamellar vesicle on nanoparticles surface forming a lipid monolayer or bilayer	Combining the intrinsic properties of silica and the bilayer	Design of biosensors
Polystyrene nanospheres	Adsorption and rupture of small unilamellar vesicle on nanoparticles surface forming a lipid monolayer or bilayer	Production of monodispersed and smooth bilayer nanosphere	Design of biosensors

Nanogels

Nanogels are colloidal microgel carriers in which cross-linked protonated polymer network binds oppositely charged drug molecules, encapsulating them into nanoparticles with a core–shell structure. The nanogel network also provides a suitable template for chemical engineering, surface modification, and vectorization.

Attempts are being made to develop novel drug formulations of nanogels with antiviral and antiproliferative nucleoside analogs in the active form of 5′-triphosphates. Notably, nanogels can improve the CNS penetration of nucleoside analogs that are otherwise restricted from passing through the blood–brain barrier. An efficient intracellular release of nucleoside analogs has been demonstrated that encourages applications of nanogel carriers for targeted drug delivery (Vinogradov 2007).

Nanogel–Liposome Combination

An appropriate assemblage of spherical nanogel particles and liposomes, termed "lipobead," combines the properties of both classes of materials and may find a variety of biomedical applications. Biocompatibility and stability, ability to deliver a broad range of bioactive molecules, environmental responsiveness of both inner nanogel core and external lipid bilayer, and individual specificity of both compartments make the liposome–nanogel design a versatile drug delivery system relevant for all known drug administration routes and suitable for different diseases with possibility of efficient targeting to different organs. New findings on reversible and irreversible aggregation of lipobeads can lead to novel combined drug delivery systems regarding lipobeads as multipurpose containers (Kazakov and Levon 2006).

Nanospheres

Hollow ceramic nanospheres (50–500 nm), created by using high-intensity ultrasound, were the first hollow nanocrystals that could be used in drug delivery (Dhas and Suslick 2005). A hollow nanocrystal of molybdenum oxide is prepared using high-intensity ultrasound to form a layer of amorphous material around a silica nanosphere. The nanosphere is then dissolved away with hydrofluoric acid and upon heating the shell crystallizes into a single hollow nanocrystal. TEM studies on the hollow ceramic materials indicate the formation of dispersed free spheres with a hollow core.

Nanotubes

Micro- and nanotubes or microstructures that resemble tiny drinking straws are alternatives that might offer advantages over spherical nanoparticles for some applications. Tubular structure of nanoparticles is highly attractive due to their structural attributes, such as the distinctive inner and outer surfaces, over conventional spherical nanoparticles.

When a PEG-silane is attached to the silica nanotubes, adsorption of IgG immunoglobulins is strongly suppressed relative to nanotubes that do not contain the attached PEG. This has potential usefulness for the delivery of biopharmaceuticals. A payload can be incorporated into the nanotubes by either covalent bonding or other chemical interactions between the payload and the inside walls of the nanotubes. For some applications, it might be useful to fill the nanotubes with the payload and then to apply caps to the nanotubes to keep the payload encapsulated. Uncapping and release of payload can be triggered by a biochemical signal.

Inner voids of nanotubes can be used for capturing, concentrating, and releasing species ranging in size from large proteins to small molecules. Distinctive outer surfaces can be differentially functionalized with environment-friendly and/or probe molecules to a specific target. By combining the attractive tubular structure with magnetic property, the magnetic nanotube can be an ideal candidate for the multifunctional nanomaterial toward biomedical applications, such as targeting drug delivery with MRI capability. Magnetic silica-iron oxide composite nanotubes have been successfully synthesized and shown to be useful for magnetic-field-assisted chemical and biochemical separations, immunobinding, and drug delivery.

Carbon Nanotubes for Drug Delivery

Carbon nanotubes (CNTs) are ready-made, strong, electrically useful microscopic tubes that form naturally in soot from sheets of carbon atoms. Various proteins adsorb spontaneously on the sidewalls of CNTs, enabling protein–nanotube conjugates. Proteins can be readily transported inside various mammalian cells via the endocytosis pathway with CNTs acting as the transporter. CNTs are useful for future in vitro and in vivo protein delivery applications. CNTs, used as liquid-filled nanoparticles, act as absorption enhancers and improve the bioavailability of erythropoietin to 11.5 % following administration into the small intestinal in experimental animals (Venkatesan et al. 2005).

CNTs can pierce cell membranes like tiny needles without damaging the cell. If proteins or nucleic acids are attached to the nanotubes, they can also go right through the cell membrane. CNTs can also carry small pharmaceutical molecules such as antibiotics or cancer drugs directly into cells and have been successfully used to inject antifungal agents into cells (Wu et al. 2005). It is also possible to attach two agents to nanotubes enabling combination therapies or to trace the uptake of a drug by adding a marker.

CNTs can be formed into nanopipettes by tapering the diameter from 700 nm to only a few nanometers with central channels that could sense chemicals at very specific locations and eventually deliver tiny amounts of fluids under the skin. Dense arrays of nanopipettes could be used for drug delivery.

CNT–Liposome Conjugates for Drug Delivery into Cells

CNTs can be used as carriers for drug delivery due to their facile transport through cellular membranes. However, the amount of loaded drug on a CNT is rather small; therefore, liposomes are employed as a carrier of a large amount of drug. In a CNT–liposome conjugate (CLC) drug delivery system, drug-loaded liposomes are covalently attached to CNT so that a high dose of the drug can be delivered into cells without potential adverse systemic effects when administered with CNTs

without liposomes (Karchemski et al. 2012). This system is expected to provide versatile and controlled means for enhanced delivery of one or more agents that can be stably conjugated with liposomes.

Lipid–Protein Nanotubes for Drug Delivery

Nanotubes for drug or gene delivery applications can be developed with open or closed ends. The nanotubes could be designed to encapsulate and then open up to deliver a drug or gene in a particular location in the body. This can be achieved by manipulating the electrical charges of lipid bilayer membranes and microtubules (MT) from cells. Synchrotron X-ray scattering and electron microscopy studies of self-assembly of cationic liposome-MT complexes show that vesicles either adsorb onto MTs, forming a "beads on a rod" structure, or undergo a wetting transition and coat the MT. Tubulin oligomers then coat the external lipid layer, forming a tunable lipid–protein nanotube. The beads on a rod structure are a kinetically trapped state. The energy barrier between the states depends on the membrane bending rigidity and charge density. The inner space of the nanotube in these experiments measures about 16 nm in diameter, and the whole capsule is about 40 nm in diameter (Raviv et al. 2007; Safinya et al. 2011). By controlling the cationic lipid/tubulin stoichiometry, it is possible to switch between two states of nanotubes with either open ends or closed ends with lipid caps, a process that forms the basis for controlled chemical and drug encapsulation and release. Taxol is one type of drug that can be delivered with these nanotubes.

Halloysite Nanotubes for Drug Delivery

Halloysite is a natural clay material typically used in ceramics. Some clay reserves contain halloysite in the form of naturally occurring nanotubes that are approximately 10–100 nm in internal diameter and vary in length from a few hundred nanometers to several micrometers. Halloysite nanotubes can be loaded with drugs for sustained release, extending the effective life of drugs as they migrate out of the tubes over time. Once loaded, these tubes can also be encapsulated to further influence the rate of elution. This enables alteration of the drug release profile and extends the effectiveness of drugs without increasing potency. Compared to CNTs, halloysite nanotubes are far less expensive and have an extraordinarily large surface area. This feature promises significant advantages for drug delivery applications since surface area contact allows for greater control of drug loading and elution profiles.

Loaded nanotubes can also be combined with other technologies for noninvasive activation. Nanotubes can be coated with nanomagnetic material that can subsequently be heated selectively and noninvasively using specific electromagnetic

energy. Heating can thus provide elution on demand. The benefits of using naturally occurring halloysite material for specific drug delivery applications are longer delivery times, more control of the drug release profile, and improved safety profiles. This technology can be applied to several product platforms, including transdermal drug delivery and drug-loaded wound care products. Transdermal delivery with halloysite nanotubes can enable a more controlled elution profile with several potential benefits:

- Eliminates the high initial delivery rate and improves the safety profile, particularly with drugs such as stimulants or hormones.
- More uniform delivery can result in better maintenance of the effective clinical dose.
- Less drug loading is required per patch. Since much of the drug is discarded when the patch is removed, this can lead to reduced costs.

Wound care products range from simple bandages to long-term treatments to promote healing and reduce the chances of infection and scarring. Drugs loaded into halloysite tubes and embedded into the base layer of a bandage can be released over an extended time period. This increases the duration of drug effectiveness and reduces the frequency with which a bandage needs to be changed. This novel delivery form can provide new dosage formulations with several advantages:

- Linear release ensures maintenance of clinically effective doses.
- Compliance and ease of use; longer elution times mean fewer bandage changes.
- Uniformity of drug delivery: elution from halloysite.

Nanocochleates

Cochleate, a lipid-based delivery system, is formed as a result of interaction between cations, e.g., Ca^{2+} and negatively charged phospholipids such as phosphatidylserine. Cochleates are stable precipitates with a unique structure consisting of a large, continuous, solid, lipid bilayer sheet rolled up in spiral, with no internal aqueous space. They are nontoxic and noninflammatory and have been used as vehicles for oral and parenteral delivery of protein and peptide antigens.

Smart Pharmaceuticals™ (BioDelivery Sciences International, BDSI) is based on Bioral™ technology, which uses nanocochleates and allows biopharmaceutical manufacturers to offer biologically active compounds with unparalleled convenience, shelf-life, and reduced side effects. This drug delivery technology has been applied to generic, off-patent injectable drugs to make them patent-protected oral drugs. The company has developed food processing technology to encochleate sensitive and easily degraded nutrients (beta-carotene, antioxidants, and others) for addition to processed food and beverages. Nanoencochleation's all-natural process

encochleates and preserves essential nutrients like antioxidants into a protected "shell" for in high-temperature/pressure canning and bottling applications.

Bioral® technology was used to encapsulate and deliver a siRNA therapeutic in a mouse model of influenza. The siRNA targets critical gene segments shared by avian influenza (H5N1). A single intranasal dose of encochleated siRNA administered 4 h after influenza exposure reduced virus titers in the lung by 200 times. It was 25 times more effective in reducing the virus than intravenous delivery as siRNA is destroyed rapidly when introduced into the blood directly.

Nanobiotechnology and Drug Delivery Devices

There is a need to improve devices introduced into the human body. Some drug delivery devices are implanted in the body for release of therapeutic substances. The lining of these devices can be improved by nanotechnology. For example, implants can be coated by nanolayers of polymers.

Nanoencapsulation

The Nanocoat™ process (Nanotherapeutics Inc) is a patented, solvent-free encapsulation system for coating micron and submicron size powders. Adapted from commercial fluidization systems, a core nanoparticle/microparticle is excapsulated with a thin layer of a coating material, such as a surfactant or a biodegradable polymer. The coating may be applied to slow the rate of release of an active component, improve the dispersion/flow properties, or increase the absorption into the systemic circulation. The Nanocoat™ process has been shown to provide a method of producing complete, or continuous, coated drug particles of high encapsulation efficiency while requiring minimal processing. The process also has several advantages over current techniques including:

- It is a fast process with run-times on the order of minutes.
- A variety of coating materials can be used, making it possible to produce films from materials with proven biocompatibility.
- It is a dry, solvent-free technique that can be conducted under a sterile cGMP conditions.
- Particle agglomeration/adhesion can be minimized by applying coatings that affect the bonding nature and electrostatic charge on the surface.

Formation of microcapsules by depositing coatings onto the nanoparticle surface will make it possible to control drug release kinetics by (a) diffusion of the drug though a polymeric coating and (b) degradation of a biodegradable polymer coating on the drug particles, releasing the core drug material.

GeneSegues' nanocapsules are designed using a flexible formulation process and have the following characteristics:

- The drug is condensed to a small molecule <50 nm in diameter.
- The drug is fully encapsulated in a stable, controlled-release capsule.
- Ability to carry large or small molecules.
- Capsule coating can be made of ligands for receptor-mediated targeted delivery to different organs, tissues, and cells.
- Choices of route of administration include topical application, tablets, intravenous, or via devices.

Nanotechnology-Based Device for Insulin Delivery

One of the main aims of insulin therapy for diabetes is to appropriately mimic physiological insulin secretion levels and their correlation with glucose concentration in healthy individuals. A nanoscale device with channels and insulin monomers/dimers enclosed was proposed that can sense the increase in glucose levels and release monomeric insulin through channels in the nanocapsule (Koch et al. 2006). Ideally, insulin dimers would be blocked from passage, which will provide physiologically relevant insulin monomers to bind to the insulin receptor. Upon return of glucose levels to basal levels, the channels will close and insulin passage is stopped. Developments in functionalized nanocontainers will enable glucose-sensitive receptacles to be engineered. Such devices could be used to provide new therapeutic approaches in insulin treatment.

Nanoporous Materials for Drug Delivery Devices

Nanoporous materials with ordered and controlled pore structures, high surface area, and pore volume are particularly suited for implantable drug delivery systems. Considerable progress has been made in electrochemically engineered nanopores/nanotube materials such as nanoporous alumina and nanotubular titanium (Losic and Simovic 2009). Nanoporous devices can be used for cell encapsulation in hormonal therapy. Biosensors mounted on these devices can be used for noninvasive signal detection.

Nanopore Membrane in Implantable Titanium Drug Delivery Device

Implanted titanium drug delivery devices using silicon nanopore membranes can control the release by diffusion of an encapsulated drug at a nearly constant rate. Nearly zero-order drug kinetics can be achieved over long periods of time. Such nanodevices are suitable for the delivery of protein and peptide drugs and avoid the poor pharmacokinetics associated with injections, providing an optimized method

of delivery for these compounds. The drug can be formulated as a dry powder or a concentrated suspension and maintains its stability. The drug is protected from the immunological reaction of the body by the nanopore membrane, which releases the drug but excludes entry of unwanted cells.

Measuring the Permeability of Nanomembranes

In order to design molecular transport systems effectively, one needs to know how big the pores in the vehicle's membranes are and how easily the contents can pass through them. This has proved quite difficult. A method for determining the permeability of thin films has been developed. A molecular beacon immobilized inside a porous silica particle that is subsequently encapsulated within a thin film can be used to determine the size of DNA that can permeate through the film (Johnston and Caruso 2005). Using this technique, it has been determined that over 3 h, molecules larger than 4.7 nm do not permeate 15-nm thick polyelectrolyte multilayers and after 75 h molecules larger than 6 nm were excluded. This technique has applications for determining the permeability of films used for controlled drug and gene delivery. A molecular beacon made from single DNA strands has been used to measure how easily DNA or genes can pass through the wall of drug delivery particles.

The beacons used are single DNA strands which have a light-emitting molecule (a fluorophore) at one end and a quencher at the other. The DNA strand self-assembles so that the two end segments pair up, forming a loop in the center – much like the shape of a round-bottomed flask. This is the closed molecular beacon. When the beacon is closed, the fluorophore on one end of the DNA strand is close to the "quencher" on the other end, which stops the fluorophore from giving off light. To determine the permeability of the capsule, the molecular beacons are placed inside the delivery vehicle. If DNA passes through the capsule wall, the beacon opens and the fluorophore emits light. So when DNA passes through the capsule, the beacon is switched "on." If no DNA passes through the capsule, the beacon remains switched off. This technique can be used in the design of intelligent drug delivery systems which can transport medicine to target locations and release the contents in a controlled way.

Nanovalves for Drug Delivery

A nanovalve that can be opened and closed at will to trap and release molecules could be used as a drug delivery system (Nguyen et al. 2005). This nanovalve consists of moving parts – switchable rotaxane molecules that resemble linear motors – attached to a tiny piece of glass (porous silica), which measures about 500 nm. It is big enough to let molecules in and out, but small enough so that the switchable rotaxane molecules can block the hole. The valve is uniquely designed so one end attaches to the opening of the hole that will be blocked and unblocked, and the other end has the switchable rotaxanes whose movable component blocks the hole in the

down position and leaves it open in the up position. The researchers used chemical energy involving a single electron as the power supply to open and shut the valve, and a luminescent molecule that allows them to tell from emitted light whether a molecule is trapped or has been released. The nanovalve is much smaller than living cells. A nanovalve combined with biomolecules could be inserted into a cell and activated by light to release a drug inside a cell.

Switchable rotaxanes are molecules composed of a dumbbell component with two stations between which a ring component can be made to move back and forth in a linear fashion. Switchable rotaxanes have been used in molecular electronics and are now being adapted for use in the construction of artificial molecular machinery. Further research will test the size hole that can be blocked to see whether larger molecules such as enzymes can be transported inside the container.

Nanochips for Drug Delivery

MicroCHIPS Inc (Bedford, MA) is working on a silver dollar-size device to implant under a patient's skin or in the abdomen that would provide tiny, precise doses of hormones, pain medication, or other pharmaceuticals. The chips, made of silicon or polymer, feature hundreds of tiny micromachined wells that can be loaded with a mixture of medicines. A microcontroller could release small amounts of different chemicals on a customizable schedule. Or biosensors could trigger releases by detecting blood sugar levels or other biochemical conditions. If approved, such a device could provide diabetics with doses of insulin so that they could forgo daily injections for as much as a year. Or it could help liberate AIDS patients from following complicated daily regimens of multiple medications. To more closely imitate how the body releases hormones, the device could dispense compounds such as estrogen in periodic bursts.

Products currently in development include external and implantable microchips for the delivery of proteins, hormones, pain medications, and other pharmaceutical compounds. A clinical trial of an implantable microchip for delivery of human parathyroid hormone fragment ($hPTH_{1–34}$) has been conducted successfully in osteoporotic postmenopausal women. Potential advantages of these microchips include small size, low power consumption, absence of moving parts, and the ability to store and release multiple drugs or chemicals from a single device.

Nanobiotechnology-Based Transdermal Drug Delivery

Introduction

Transdermal drug delivery is an approach used to deliver drugs through the skin for therapeutic use as an alternative to oral, intravascular, subcutaneous, and transmucosal routes. Technical details are described in a special report on transdermal drug

delivery (Jain 2012d). Nanoparticles and nanoemulsions have better skin penetration than larger particles.

There is experimental evidence for the potential of nanoparticles as delivery vectors for antigens and DNA for the purpose of transdermal vaccination protocols. Fluorescent particles ranging in size and charge were applied to the surface of full thickness pig skin in a diffusion chamber, and the receptor fluid was assayed to determine permeation (Kohli and Alpar 2004). Fluorescence microscopy was used to visualize the skin after experiments. The results showed that only 50- and 500-nm particles that were negatively charged were able to permeate the skin, indicating that negative particles with sufficient charge may be ideal carriers for this purpose.

Delivering genes and drugs within cells with devices approaching the nanoscale allows for new levels of precision and minimal damage to cells. Nanopatches can be used to target immunologically sensitive cells for DNA vaccination of malaria and allergies. This technology will also enable pain-free and needle-free immunotherapy of asthma.

The focus in this section is on the use of transdermal route for systemic delivery of therapeutics. Use of nanobiotechnology to improve skin penetration of drugs used for treatment of skin disorders will be described separately later in this book.

Delivery of Nanostructured Drugs from Transdermal Patches

Nanobiotechnology has been applied for the painless transdermal delivery of vaccines, peptide hormones, and other drugs. The patches are structured on the skin side with microprotrusions, which hold the drugs to be delivered. The protrusion face of the patch is applied to the skin where they cross the outer surface layer of the skin only reaching as far as the interstitial space avoiding nerves and blood vessels. In this interstitial space, the nanostructured drugs are released from the surface of the protrusions, and as the biocompatible polymer biodegrades, the drugs are released continuously from the body of the protrusions. The nanostructured drugs are either taken up by the cells of the immune system (for vaccination applications) or flow through the interstitial fluid to other compartments in the body.

Effect of Mechanical Flexion on Penetration of Buckyballs Through the Skin

Normally bucky amino clusters form spherical clusters that are up to 12 times larger than the width of the intercellular gaps in skin. In one study, confocal microscopy depicted dermal penetration of fullerene-substituted phenylalanine (Baa) derivative of a nuclear localization peptide at 8 h in skin flexed for 60 and 90 min, whereas Baa-Lys(FITC)-NLS, but did not penetrate into the dermis of unflexed skin until 24 h (Rouse et al. 2007). Transmission electron microscopy

analysis revealed fullerene–peptide localization within the intercellular spaces of the stratum granulosum. This study shows that repetitive movement can speed the passage of nanoparticles through the skin.

Ethosomes for Transdermal Drug Delivery

Ethosomes – soft, malleable vesicles with size ranging from 30 nm to a few microns – form the basis of Ethosome Delivery System (Novel therapeutic Technologies). Ethosomal systems were found to be significantly superior at delivering drugs through the skin in terms of both quantity and depth when compared to liposomes and to many commercial transdermal and dermal delivery systems. Visualization by dynamic light scattering showed that ethosomes could be unilamellar or multilamellar through to the core. These novel delivery systems contain soft phospholipid vesicles in the presence of high concentrations of ethanol. Ethosomal systems are sophisticated conceptually but characterized by simplicity in their preparation, safety, and efficiency – a rare combination that can expand their applications.

Because of their unique structure, ethosomes are able to encapsulate and deliver through the skin highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well as cationic drugs such as propranolol and trihexyphenidyl. Results obtained in a double-blind two-armed randomized clinical study showed that treatment with the ethosomal acyclovir formulation significantly improved all the evaluated parameters (Godin and Touitou 2003).

Ethosomes penetrate cellular membrane releasing the entrapped molecule within cells. Studies focused on skin permeation behavior of fluorescently labeled bacitracin from ethosomal systems through human cadaver and rat skin demonstrated that the antibiotic peptide was delivered into deep skin layers through intercorneocyte lipid domain of stratum corneum (Godin and Touitou 2004). Ethosomal delivery systems could be considered for the treatment of a number of dermal infections, requiring intracellular delivery of antibiotics, whereby the drug must bypass two barriers: the stratum corneum and the cell membrane. Ethosomal formulation of testosterone could enhance testosterone systemic absorption and also be used for designing new products that could solve the weaknesses of the current testosterone replacement therapies (Ainbinder and Touitou 2005).

Advantages of ethosomes over other transdermal delivery systems are:

- Enhanced permeation
- Platform for the delivery of large and diverse group of drugs including peptides and very lipophilic molecules
- Safe and approved components
- Passive, noninvasive delivery system
- Available for immediate commercialization
- High patient compliance
- High cost to benefit ratio

NanoCyte Transdermal Drug Delivery System

NanoCyte drug delivery system (NanoCyte Inc) is based on a sophisticated injection system developed by the sea anemone during million years of evolution. The NanoCyte natural substance is extracted from aquatic invertebrates. Each microcapsule contains a coiled microscopic nanotube, which unfolds on activation – a process whereby high pressure of 200 atmospheres is developed within the microcapsule. The long thin nanotube evaginates out of the microcapsule and penetrates the skin at an acceleration of 40,000 g to deliver the drug efficiently in a fraction of a second into the epidermis skin layer. NanoCyte can be formulated as a suspension, lotion, cream, or a stick. NanoCyte can also be activated after attaching to an adhesive patch. Advantages of this system include:

- Immediate intradermal delivery
- Device-less active delivery
- Painless administration
- Avoidance of large dosages and side effects
- Treatment of large skin areas
- Multipoint penetration using nanoinjectors
- Ease of use

Safety Issues of Applications of Nanomaterial Carriers on the Skin

The European Commission has requested the Scientific Committee on Consumer Products (SCCP) to prepare an opinion on "Safety of Nanomaterials in Cosmetic Products." The preliminary version of the opinion can be found online (http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_099.pdf). The results obtained with nanosized delivery systems were not consistent. The following list of potential properties was considered that are relevant to transdermal drug delivery:

- Nanomaterial constituents (such as lipids or surfactants) may act as penetration enhancers by penetrating individually into the stratum corneum (after particle disruption on skin surface) and subsequently altering the intercellular lipid lamellae within this skin layer.
- Nanomaterials may serve as a depot for sustained release of dermally active compounds.
- Nanomaterials may serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.

Transdermal Administration of Lipid Nanocapsules

Due to their small size, lipid nanocapsules (LNC) are a promising as a drug delivery system – as injectable or an oral or by transdermal route. Biocompatible ibuprofen LNC has been developed with a particle size of ~50 nm. Pain relief after intravenous administration of ibuprofen is prolonged by at least 2 h when administering LNC formulation. LNCs can also be used as a transdermal drug delivery system using ibuprofen as a model drug. A comparison to other lipid nanocarriers such as solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) and polymeric nanocarriers has been made. Polymeric carriers had fourfold higher accumulation in the skin compared to that of the LNC and twice the accumulation of SLN and NLC (Abdel-Mottaleb et al. 2011). These results would suggest that the LNC can be considered as efficient as SLN and NLC for the transdermal drug delivery whereas polymeric nanoparticles are more suitable for localized drug delivery to the skin.

Transdermal Nanoparticle Preparations for Systemic Effect

Nanoparticles have been used to facilitate transdermal delivery of several systemic drugs. Some examples are as follows:

$CaCO_3$–nanoparticle system successfully delivered insulin transdermally, as evidenced by a significant sustained decrease in blood glucose in normal mice and those with diabetes (Higaki et al. 2006). These results support the feasibility of developing transdermal nanoinsulin for human applications. A novel MicroArray Patch (Interstitial NS) is in preclinical development for insulin delivery using passive (diffusion) patches.

A multicenter, randomized, double-blind, placebo-controlled study showed that once-daily application of micellar nanoparticle estradiol emulsion was safe and effective in providing significant relief of vasomotor symptom frequency and severity in postmenopausal women (Simon et al. 2006). This is the basis of Estrasorb® (Novavax), a transdermal lotion containing estrogen for relief of menopausal symptoms, and Androsorb® (Novavax), a transdermal lotion containing androgen.

Indomethacin-loaded poly(*n*-butylcyanoacrylate) nanocapsules can improve the transdermal delivery of indomethacin compared to a conventional gel formulation. This might be due to their ultrafine particle size and their hydrophilic and hydrophobic surface characteristics.

Lipid nanoparticles have been used for transdermal delivery of flurbiprofen (Bhaskar et al. 2009). The bioavailability of flurbiprofen with reference to oral administration was found to increase by 4.4 times when gel formulations were applied and sustained drug release was demonstrated over a period of 24 h in studies on experimental animals.

Celecoxib, a selective cyclooxygenase-2 inhibitor, has been recommended orally for the treatment of arthritis and osteoarthritis. Long-term oral administration of celecoxib produces serious gastrointestinal side effects. It is a highly lipophilic,

poorly soluble drug with oral bioavailability of around 40 % (capsule). The skin permeation mechanism and bioavailability of celecoxib by transdermally applied nanoemulsion formulation have been investigated (Shakeel et al. 2008). Optimized oil-in-water nanoemulsion of celecoxib was prepared by the aqueous phase titration method. Fourier transform infrared spectra and differential scanning calorimeter thermogram of skin treated with nanoemulsion indicated that permeation occurred due to the disruption of epidermal lipid bilayers by nanoemulsion, which was demonstrated by photomicrograph of skin sample. The absorption of celecoxib through transdermally applied nanoemulsion gel resulted in approximately threefold increase in bioavailability as compared to oral capsule formulation. Thus, nanoemulsions are potential vehicles for enhancement of skin permeation and bioavailability of poorly soluble drugs.

Nasal Drug Delivery Using Nanoparticles

The nasal cavity is an ideal site for delivery of both locally and systemically acting drugs. Topical administration includes agents for the treatment of nasal congestion, rhinitis, sinusitis, and related allergic and other chronic conditions. Various medications include corticosteroids, antihistaminics, anticholinergics, and vasoconstrictors. The focus in recent years has been on the use of nasal route for systemic drug delivery. Intranasal route is considered for drugs which are ineffective orally, are used chronically, and require small doses and where rapid entry into the circulation is desired. The rate of diffusion of the compounds through the nasal mucous membranes, like other biological membranes, is influenced by the physicochemical properties of the compound. Impressive improvements in bioavailability have been achieved with a range of compounds.

Chitosan, a naturally occurring polysaccharide derived from chitin, is used as an absorption enhancer for transnasal drug delivery. Chitosan is bioadhesive and binds to the mucosal membrane, prolonging retention time of the formulation on the nasal mucosa. It may also facilitate absorption through promoting paracellular transport. The chitosan nasal technology can be exploited as solution, dry powders, or nanoparticle formulations to further optimize the delivery system for individual compounds. For compounds requiring rapid onset of action, the nasal chitosan technology can provide a fast peak concentration compared with oral or subcutaneous administration. Density and size of PEG coating of poly(lactic acid)-poly(ethylene glycol) (PLA-PEG) nano- and microparticles have an important effect on their transport across the nasal mucosa. PLA-PEG particles with a high PEG coating density and a small size are more significantly transported than noncoated PLA nanoparticles or PLA-PEG nanoparticles with a lower coating density.

Nanoparticles made of low molecular weight chitosan are promising carriers for nasal vaccine delivery. Compacted DNA nanoparticles, encoding cystic fibrosis transmembrane regulator gene, can be safely administered by perfusion to the nose of cystic fibrosis subjects. A double-blind, dose escalation gene therapy trial with

this technique showed evidence of vector gene transfer and partial correction of nasal potential difference that is typical for subjects with classic cystic fibrosis.

Mucosal Drug Delivery with Nanoparticles

The layers of mucus that protect sensitive tissue throughout can also prevent the entry of drugs into the body. The role of nanoparticles as drug delivery vehicles has been explored to overcome this hurdle. Cervicovaginal mucus was used for these investigations because its viscoelastic properties and mucin concentration are similar to those in many other human mucus secretions. Large nanoparticles, 200- to 500-nm in diameter, if coated with polyethylene glycol, diffused through mucus with an effective diffusion coefficient (Deff) only four- and sixfold lower than that for the same particles in water (Lai et al. 2007). In contrast, for smaller but otherwise identical 100-nm coated particles, Deff was 200-fold lower in mucus than in water. For uncoated particles 100–500 nm in diameter, Deff was 2,400- to 40,000-fold lower in mucus than in water. Much larger fractions of the 100-nm particles were immobilized or otherwise hindered by mucus than the large 200- to 500-nm particles. Thus, in contrast to the prevailing belief, these results demonstrate that large nanoparticles, if properly coated, can rapidly penetrate physiological human mucus, and they offer the prospect that large nanoparticles can be used for mucosal drug delivery.

Future Prospects of Nanotechnology-Based Drug Delivery

A desirable situation in drug delivery is to have smart drug delivery systems that can integrate with the human body. This is an area where nanotechnology will play an extremely important role. Even time-release tablets, which have a relatively simple coating that dissolves in specific locations, involve the use of nanoparticles. Pharmaceutical companies are already involved in using nanotechnology to create intelligent drug release devices. For example, control of the interface between the drug/particle and the human body can be programmed so that when the drug reaches its target, it can then become active. The use of nanotechnology for drug release devices requires autonomous device operation. For example, in contrast to converting a biochemical signal into a mechanical signal and being able to control and communicate with the device, autonomous device operation would require biochemical recognition to generate forces to stimulate various valves and channels in the drug delivery systems, so that it does not require any external control.

Subcellular or organelle-specific targeting has emerged as a new frontier in drug delivery. Nanocarriers will create the next generation of “magic bullets” that are capable of delivering a drug payload to a molecular target at a subcellular location (D’Souza and Weissig 2009). It now appears that we are on the verge of bioengineering

molecular motors for specialized applications on nanoscale. These systems might be the key to yet unsolved biomedical applications that include nonviral gene therapy and interneuron drug delivery. Examples of some potential nanotechnology-based drug delivery systems are given in the following paragraphs.

Nanomolecular Valves for Controlled Drug Release

A macroscopic valve is a device with a movable control element that regulates the flow of gases or liquids by blocking and opening passageways. Construction of such a device on the nanoscale level requires (1) suitably proportioned movable control elements, (2) a method for operating them on demand, and (3) appropriately sized passageways. These three conditions can be fulfilled by attaching organic, mechanically interlocked, linear motor molecules that can be operated under chemical, electrical, or optical stimuli to stable inorganic porous frameworks (i.e., by self-assembling organic machinery on top of an inorganic chassis). A reversibly operating nanovalve has been demonstrated that can be turned on and off by redox chemistry (Nguyen et al. 2005). It traps and releases molecules from a maze of nanoscopic passageways in silica by controlling the operation of redox-activated bistable rotaxane molecules tethered to the openings of nanopores leading out of a nanoscale reservoir. Future applications could include nanofluidic systems and the controlled release of drugs from implants with nanoscopic properties.

Nanosponge for Drug Delivery

Nanosponges are hyper-cross-linked cyclodextrin polymers nanostructured to form 3D networks and are obtained by complexing cyclodextrin with a cross-linker such as carbonyldiimidazole. They have been used to increase the solubility and stability of poorly soluble drugs. β-cyclodextrin nanosponges loaded with anticancer agent tamoxifen have been used for oral drug delivery (Torne et al. 2012). In experimental studies, tamoxifen nanosponge complex with particle size of 400–600 nm was shown to be more cytotoxic than plain tamoxifen after 24 and 48 h of incubation.

Another method for making a nanosponge uses extensive internal cross-linking to scrunch a long, linear molecule into a sphere about 10 nm in diameter. Instead of trying to encapsulate drugs in nanoscale containers, this approach creates a nanoparticle with a large number of surface sites where drug molecules can be attached. A molecular transporter attached to the nanosponge can carry it and its cargo across biological barriers into specific intracellular compartments. The transporter can deliver large molecules – specifically peptides and proteins – into specific subcellular locations. A targeting unit attached to this delivery system can deliver drugs to the surface of tumors in the lungs, brain, and spinal cord. This delivery system can be adapted to carry the chemotherapy agents for targeted delivery to tumors.

Nanomotors for Drug Delivery

Basics of nanomotors – nanometer-scale machines, which are powered by chemical reactions – have been described in Chap. 3 as molecular motors. A technique to create catalytic nanomotors uses "dynamic shadowing growth," which involves a simple modification of existing methods to allow for greater flexibility in designing desired nanomotor structures (He et al. 2007). These could be used as tools to open constricted or clogged blood vessels too small for conventional stents, or they could deliver drugs by drilling through the cell wall of an organism. The researchers looked at the hundreds of moving parts in an automobile for designing each part of a nanomotor so as to achieve a controlled, flexible range of motion for the parts to work together. After successfully using the new technique to design nanorods to rotate, they broke the symmetry of the rods to form L-shaped rods which could then be aggregated to form larger particles. Then they transformed the rod into a spiral shape so that its rotation would mimic the turning of a drill. The team used the new technique to deposit a platinum or silver catalyst on different portions of the L-shaped rods and then designed different experiments to test their ability to control the motion. In a solution of hydrogen peroxide, they captured images of the nanorods turning precisely in the directions proscribed by the catalyst depositions.