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Review

Nanoparticles in cellular drug delivery

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ABSTRACT

This review highlights the properties of nanoparticles used in targeted drug delivery, including delivery to cells as well as organelle targets, some of the known pharmacokinetic properties of nanoparticles, and their typical modifications to allow for therapeutic delivery. Nanoparticles exploit biological pathways to achieve payload delivery to cellular and intracellular targets, including transport past the blood-brain barrier. As illustrative examples of their utility, the evaluation of targeted nanoparticles in the treatment of cancers and diseases of the central nervous system, such as glioblastoma multiforme, neurovascular disorders, and neurodegenerative diseases, is discussed.

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1. Introduction

Nanotechnology is a rapidly expanding field, encompassing the development of man-made materials in the 5–200 nanometer size range. This dimension vastly exceeds that of standard organic molecules, but its lower range approaches that of many proteins and biological macromolecules (Fig. 1).

The first practical applications of nanotechnology can be traced to advances in communications, engineering, physics, chemistry, biology, robotics, and medicine. Nanotechnology has been utilized in medicine for therapeutic drug delivery and the development of treatments for a variety of diseases and disorders. The rise of nanomaterials correlates with further advances in these disciplines.

Nanoparticles appeal to scientists across many disciplines due the opportunity to engineer many properties that might otherwise be incompatible on a single device. Relevant attachments include biologically active molecules, targeting sequences, fluorescent or other imaging devices, biocompatible coatings, and others. Furthermore, the engineering of the particle backbone structure and the size and shape of the nanoparticle core provides yet another dimension of physical control that can be exerted toward the specific tailoring of function. This review focuses on applications in the cellular and intracellular delivery of therapeutic agents. We explore various types of nanoparticles (Fig. 2), ranging from ceramics to liposomes, as well as current methodologies to develop inorganic nanoparticles. A brief discussion of the pharmacokinetic parameters and specific targeting strategies of these nanoparticles follows, presenting suggestions for the mechanisms of cellular and intracellular uptake. Because of the remarkable drug delivery challenges in the central nervous system's blood-brain barrier, illustrative examples of nanoparticles in the treatment of neurological cancer, neurovascular disorders, and neurodegenerative diseases are provided.

Medical therapies have become more tailored to specific diseases and patients in recent years. Most pharmaceutical agents have primary targets within cells and tissues; ideally, these agents may be preferentially delivered to these sites of action within the cell. Selective subcellular delivery is likely to have greater therapeutic benefits. Cytosolic delivery, for instance, is desirable for drugs that undergo extensive exportation from the cell via efflux transporters such as multi-drug resistance proteins and P-glycoproteins.[1] These efflux mechanisms continuously reduce therapeutic intracellular drug concentrations. An intracellular nanoparticle, consequently, may act as a drug depot within the cell. Nanotechnology may be used to achieve therapeutic dosing via targeted

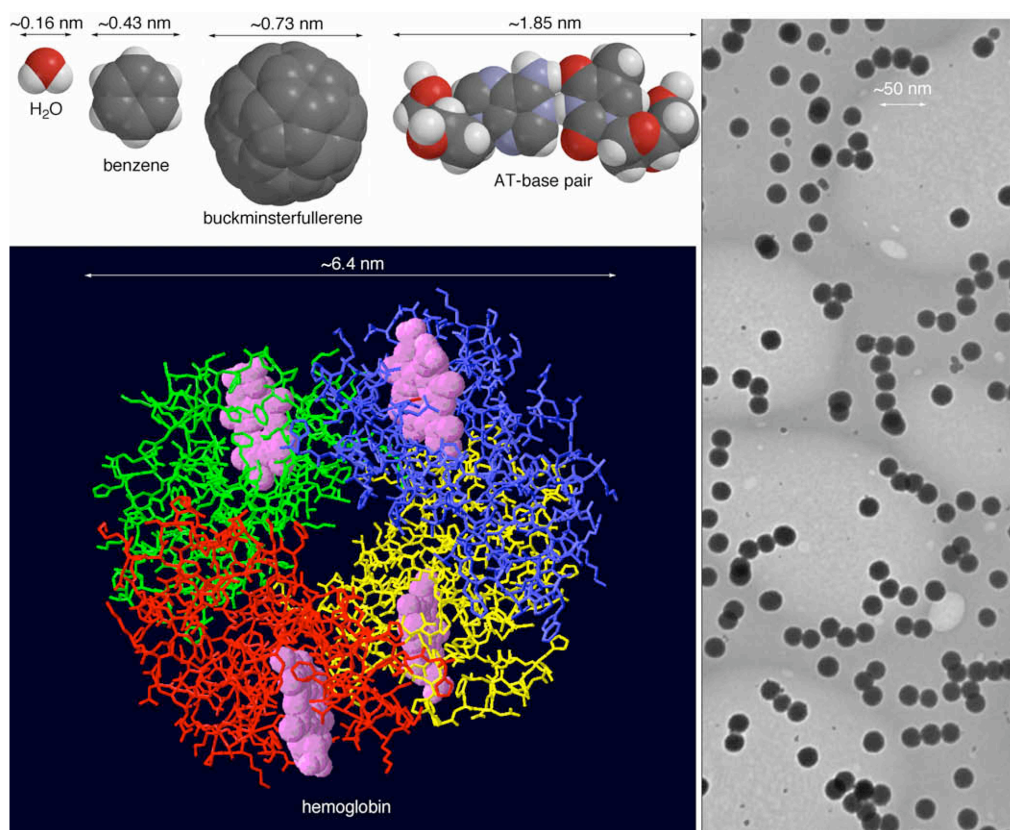

Figure 1. Sizes of organic molecules and biological macromolecules (left) in relation to silica nanoparticles (right).

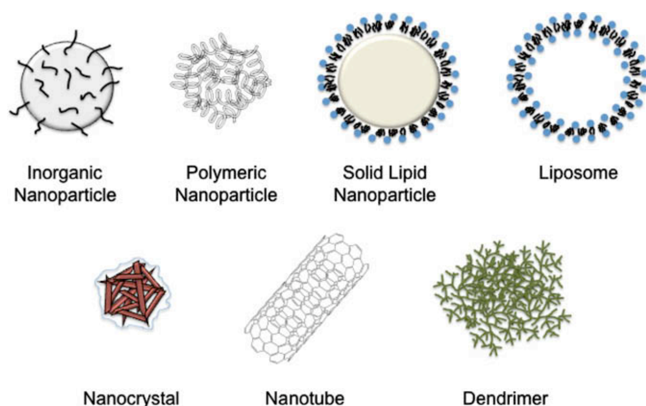

Figure 2. Various types of nanoparticles used in biomedical research and drug delivery.

therapies, establish sustained-release drug profiles, and provide an intracellular sanctuary to protect therapeutic compounds from efflux or degradation.

2. Nanoparticle types

2.1. Inorganic nanoparticles

Ceramic nanoparticles are typically composed of inorganic compounds such as silica or alumina. However, the nanoparticle core is not limited to just these two materials; rather, metals,[2–4] metal oxides,[5–12] and metal sulfides[13–15] can be used to produce a myriad of nanostructures with varying size, shape, and porosity.

Generally, inorganic nanoparticles may be engineered to evade the reticuloendothelial system by varying size and surface composition. Moreover, they may be porous, and provide a physical encasement to protect an entrapped molecular payload from degradation or denaturization. Hollow silica nanoparticles have been prepared, such as calcium phosphate-based nanoshells, with surface pores leading to a central reservoir.[16,17] In contrast, mesoporous silica materials contain a complex 'worm-like' network of channels throughout the interior of the solid nanoparticles.

Mobil first discovered the mesoporous silica-based nanomaterial MCM-41 in 1992.[18] Since that time, there has been a great interest in their surface functionalization and morphology control. For example, Vallet-Regi et al. studied the viability of this material as a drug delivery system.[19] By using C_{12}-trimethylammonium bromide versus C_{16}-trimethylammonium bromide in the self-assembly of these nanoparticles, the distribution of pore sizes—a parameter that determines the release kinetics of the drug payload as studied by Botterhuis et al.[20]—was tuned from a center at 1.8 nm to 2.5 nm. As a further extension of this work, ibuprofen was introduced into the pores of these nanomaterials at a drug-to-MCM-41 weight ratio of approximately 3:7, as determined by thermogravimetry. These loaded MCM-41 particles were then subjected to a simulated body fluid and determined to be potentially viable drug delivery systems.[19] This work demonstrated that mesoporous silica materials could be used to deliver relatively large doses of drug in a controlled manner.

The drug delivery characteristics of mesoporous materials have also been modified via reversible capping of the surface pores. Utilizing the MCM-41 scaffold, Lai et al. developed chemically removable cadmium sulfide (CdS) nanoparticle caps for use as a stimuli-responsive releasing agent for neurotransmitters and drugs.[21] Silica MCM-41 nanoparticles were modified using 2-(propyldisulfanyl)ethylamine and capped with water-soluble mercaptoacetic acid-derivatized CdS nanocrystals via an amidation reaction. The disulfide linkages were shown to be labile and chemically cleavable by disulfide reducing agents. The release rate from mesoporous silica materials was dependent on the rate of CdS cap removal. Analogous methods for controlling the release of drug payloads from silica nanomaterials have been employed by the same group using dendrimer and magnetic caps.[22,23] It is relatively easy to modify the surfaces of these particles with unique functionalities via a variety of chemical transformations. As the CdS example illustrated with modifications of 2-(propyldisulfanyl)ethylamine, several functional groups can be introduced onto the surface of inorganic nanoparticles, ranging from saturated and unsaturated hydrocarbons to carboxylic acids, thiols, amines, and alcohols. Inorganic nanoparticles are relatively stable over broad ranges of temperature and pH, yet their lack of biodegradation and slow dissolution raises safety questions, especially for long-term administration.

2.2. Polymeric nanoparticles

Most polymeric nanoparticles are biodegradable and biocompatible, and have been adopted as a preferred method for nanomaterial drug delivery. They also exhibit a good potential for surface modification via chemical transformations, provide excellent pharmacokinetic control, and are suitable for the entrapment and delivery of a wide range of therapeutic agents. Pertinent nanoparticle formulations include those made from gelatins, chitosan, poly(lactic-co-glycolic acid) copolymer, polylactic acid, polyglycolic acid, poly(alkylcyanoacrylate), poly(methylmethacrylate), and poly(butyl)cyanoacrylate. Furthermore, polymer-based coatings may be functionalized onto other types of nanoparticles to change and improve their biodistribution properties. The biologically inert polymer poly(ethylene glycol) (PEG) has been covalently linked onto the surface of nanoparticles.[24–26] This polymeric coating is thought to reduce immunogenicity, and limit the phagocytosis of nanoparticles by the reticuloendothelial system, resulting in increased blood levels of drug in organs such as the brain, intestines, and kidneys.[27,28]

The US Food and Drug Administration (FDA) has approved biodegradable polymeric nanoparticles, such as PLA and PLGA, for human use. They may be formulated to encapsulate several classes of therapeutic agents including, but not limited to, low molecular weight compounds.[29] Moreover, polymeric nanoparticles have been applied in gene therapy to breast cancer cells, resulting in antiproliferative effects.[30] The polymer matrix prevents drug degradation and may also provide management of drug release from these nanoparticles. Varying the drug-to-polymer ratio and molecular weight and composition of the polymer can modify the extent and level of drug release.[31] The surface properties of these polymeric nanoparticles are also a vital component of their targeting characteristics. Since nanoparticles come into direct contact with cellular membranes, their surface properties may determine the mechanism of internalization and intracellular localization.[32]

The general biocompatibility and biodegradation profiles of polymeric nanoparticles are attractive; this is especially true with formulations that require more chronic dosing, perhaps in contrast to many inorganic nanoparticles. Practically, large-scale production and manufacturing remains an issue with polymeric nanoparticles. For instance, PLGA nanoparticles are mostly formulated using a double emulsion solvent evaporation system, utilizing water and oil with poly(vinyl alcohol) (PVA) as an emulsifier.[31,32]

2.3. Solid lipid nanoparticles

Solid lipid nanoparticles are lipid-based submicron colloidal carriers. They were initially designed in the early 1990s as a pharmaceutical alternative to liposomes and emulsions. In general, they

are more stable than liposomes in biological systems due to their relatively rigid core consisting of hydrophobic lipids that are solid at room and body temperatures, surrounded by a monolayer of phospholipids.[33–35] These aggregates are further stabilized by the inclusion of high levels of surfactants. Because of their ease of biodegradation, they are less toxic than polymer or ceramic nanoparticles. They have controllable pharmacokinetic parameters and can be engineered with three types of hydrophobic core designs: a homogenous matrix, a drug-enriched shell, or a drug-enriched core.

Two primary production methods exist, including a high-pressure homogenization technique devised by Müller and Lucks[36] and a microemulsion technique pioneered by Gasco.[37] It has been demonstrated that the compound payload exits the hydrophobic core at warmer temperatures; conversely, the compound payload enters the hydrophobic core at cooler temperatures.[38] These principles are used to load and unload solid lipid nanoparticles for the delivery of therapeutic agents, taking advantage of recent techniques to selectively produce hypo- and hyperthermia. Additionally, the amount of surfactant used during production contributes to the release profile of the drug payload. Solid lipid nanoparticles can be used to deliver drugs orally, topically, or via inhalation.

2.4. Liposomes

Liposomes are concentric bilayered vesicles with an surrounded by a phospholipid membrane. They are related to micelles which are generally composed of a monolayer of lipids. The amphiphilic nature of liposomes, their ease of surface modification, and a good biocompatibility profile make them an appealing solution for increasing the circulating half-life of proteins and peptides. They may contain hydrophilic compounds, which remain encapsulated in the aqueous interior, or hydrophobic compounds, which may escape encapsulation through diffusion out of the phospholipid membrane. Liposomes can be designed to adhere to cellular membranes to deliver a drug payload or simply transfer drugs following endocytosis.[39–42]

Despite a relatively long history of investigation, liposomes have not yet made a significant medical impact; however, they have been extensively employed in cosmetic products. The first formulation was prepared in 1986 by the Christian Dior laboratories in collaboration with the Pasteur Institute.[42] Presumably, the lack of widespread medical impact is due to their limited biological stability. Longer liposome circulatory residency times have been demonstrated upon functionalization with PEG. These longer residency times may allow for a better control of therapeutic drug delivery.

2.5. Nanocrystals

Nanocrystals are aggregates of molecules that can be combined into a crystalline form of the drug surrounded by a thin coating of surfactant. They have extensive uses in materials research, chemical engineering, and as quantum dots for biological imaging,[43–46] but less so in nanomedicine for drug delivery.

A nanocrystalline species may be prepared from a hydrophobic compound and coated with a thin hydrophilic layer. The biological reaction to nanocrystals depends strongly on the chemical nature of this hydrophilic coating. The hydrophilic layer aids in the biological distribution and bioavailability, and prevents aggregation of the crystalline drug material. These factors combine to increase the efficiency of overall drug delivery.[47,48] High dosages can be achieved with this formulation, and poorly soluble drugs can be formulated to increase bioavailability via treatment with an appropriate coating layer. Both oral and parenteral deliveries are possible, and the limited carrier, consisting of primarily the thin coating of surfactant, may reduce potential toxicity.[49] A drawback, however, is that the stability of nanocrystals is limited. Moreover, this technique requires crystallization; some therapeutic compounds may not be easily crystallized.

2.6. Nanotubes

Nanotubes are self-assembling sheets of atoms arranged in tubes. They may be organic or inorganic in composition and can be produced as single- or multi-walled structures. A popular version of a nanotube involves the use of soluble fullerene derivatives, such as C_{60}. Nanotubes have large internal volumes and the external surface can be easily functionalized. While they are potentially promising for pharmaceutical applications, human tolerance of these compounds remains unknown, and toxicity reports are conflicting. It has been demonstrated that nanotubes are acutely toxic and may cause cellular death via an oxidative-stress pathway.[50–52] Extensive research into the biocompatibility and toxicity of nanotubes remains ongoing.

2.7. Dendrimers

Dendrimers are polymer-based macromolecules formed from monomeric or oligomeric units, such that each layer of branching units doubles or triples the number of peripheral groups. The void area within a dendrimer, the extent of its branching, its ease of modification and preparation, and size control offer great potential for drug delivery. Dendrimers generally have a symmetrical structure, with the potential to create an isolated 'active site' core area through chemical functionalization. Modification of the degree of branching may allow for encapsulation of a molecule within this structure.[53] For example, a dendrimer may become water-soluble when its end-groups are functionalized with hydrophilic groups, such as carboxylic acids. Thus, water-soluble dendrimers may be designed with internal hydrophobicity, suitable for the incorporation of a hydrophobic drug. The frequently used genetic transfection agent Polyfect consists of dendrimer molecules radiating from a central core. Amino groups at the terminal ends of the dendrimer branches are positively-charged at physiological pH, therefore interacting with the negatively-charged phosphate groups of nucleic acids.[54] However, dendrimers require further improvements in cytotoxicity profiles, biocompatibility, and biodistribution.

3. Nanoparticle synthesis and conjugation methodologies

3.1. Gold nanoparticles

The preparation of gold nanoparticles commonly involves the chemical reduction of gold salts in aqueous, organic, or mixed solvent systems. However, the gold surface is extremely reactive, and under these conditions aggregation occurs. To circumvent this issue, gold nanoparticles are regularly reduced in the presence of a stabilizer, which binds to the surface and precludes aggregation via favorable cross-linking and charge properties. Several stabilizers exist for passivation of the gold nanoparticle surface, including citrate,[55] thiol-containing organic groups,[56] encapsulation within microemulsions,[57] and polymeric coatings.[58] In particular, gold nanoparticles may be encrusted with biomolecules, with exciting prospects in biological sensing and imaging. Several synthetic strategies exist, such as the two phase liquid–liquid method initially described to create metal colloidal suspensions by Faraday in 1857.[59] Faraday reduced an aqueous gold salt with phosphorous in carbon disulfide to obtain a ruby-colored aqueous suspension of colloidal gold particles. The Brust–Schiffrin method further opti-

mized this two phase liquid–liquid system with gold salts being transferred from water to toluene using tetraoctylammonium bromide as the phase transfer reagent, with reduction by aqueous sodium borohydride in the presence of dodecanethiol.[60] Using modifications of this method, gold nanoparticles have been synthesized with numerous biomolecular coatings.[61,62] The resulting gold nanoparticles have biological applications; for instance in the detection of polynucleotides via hybridization to oligonucleotides appended on the nanoparticle surface.[63]

3.2. Carbon nanotubes

Carbon nanotubes were initially discovered in 1991 in cathode deposits following arc evaporation of graphite.[64] Shortly after this seminal report, carbon nanotubes were isolated after pyrolysis of hydrocarbons such as ethylene or acetylene over nanoparticles of iron, cobalt, or other dispersed metals.[65–67] The presence of these materials greatly influences the size profile of the developing nanotubes.[68] Sen et al. prepared multi-walled carbon nanotubes (MWNT) by pyrolysis of metallocenes such as ferrocene, cobaltocene, and nickelocene under reducing conditions; the metallocene precursor acts as a source for both metal nanoparticles and carbon.[69] Single-walled carbon nanotubes (SWNT) were prepared in a related approach using dilute hydrocarbon–organometallic mixtures.[70,71] Interestingly, pyrolysis of nickelocene in the presence of benzene at 1100 °C yields primarily MWNT. In contrast, pyrolysis of nickelocene in the presence of acetylene yields primarily SWNT, presumably due to the smaller number of carbon atoms per molecule.[72]

3.3. Layered double hydroxide nanoparticles

Layered double hydroxide nanoparticles are a comparatively new focus of study for drug delivery, gene therapy, and controlled-release agents. This interest is spurred by their low cytotoxicity and high biocompatibility.[73,74] Traditional synthetic strategies exploit coprecipitation of mixed salts in hydroxide solutions at variable or invariable pH followed by curing at an elevated temperature.[75–77] Unfortunately, these techniques frequently result in aggregated particles. Several modifications exist to tune the size and aggregation properties. The utilization of separate nucleation and curing techniques by Zhao et al. provides colloidal suspensions of layered double hydroxide nanoparticles with sizes ranging from 1 to 10 μm.[78] These sizes are quite large and may be biologically irrelevant for drug delivery. Modulating the size profile of these nanoparticles to obtain smaller drug delivery carriers necessitates a precise control of the hydrothermal treatment stage. Recent work by Xu et al. has demonstrated the synthesis of monodisperse nanoparticles with sizes ranging from 40 to 300 nm, thus approaching the range of biological compatibility.[79]

3.4. Iron oxide nanoparticles

Several synthetic strategies exist to prepare ferromagnetic iron oxide nanoparticles. In particular, a water-in-oil microemulsion system with reverse micelles has been utilized extensively.[80–82] For maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4) nanoparticles, this precipitation technique requires alkalization of a solution of metal salt with subsequent hydrolysis in microemulsions. Additionally, biosynthetic routes exist utilizing magnetic bacteria; the resulting nanoparticles typically range from 50 to 100 nm in diameter.[83–85] The synthesis of iron oxide nanoparticles has also been realized by sonochemical decomposition of iron pentacarbonyl,[86,87] thermal decomposition of other iron complexes,[88,89] and by thermal decomposition of iron pentacarbonyl followed by oxidation.[90] When optimized, these methods may afford monodisperse nanoparticles with sizes ranging from 3 to 20 nm for magnetite and 4 to 16 nm for maghemite.[89,90] Furthermore, iron oxide nanoparticles also display fairly easy surface modification capabilities. Iida et al. functionalized the surface of commercially-available 3 nm maghemite nanoparticles using 3-aminopropyltriethoxysilane.[91a] This presents an attractive prospect for direct drug or biomolecule payload attachment. Recently, ultrasmall, peptide-coated magnetite nanoparticles were used to target integrin-rich tumor cells.[91b]

3.5. Calcium phosphate nanoparticles

Calcium phosphates, also known as hydroxyapatite, represent the majority of the inorganic matter of human hard tissue such as bone and teeth. Accordingly, calcium phosphate nanoparticles display excellent biocompatibility.[92,93] These nanoparticles may be synthesized by a myriad of methods including wet chemical routes,[94] solid-state reactions and hydrothermal reactions at elevated temperature,[95] biosynthetic routes,[96] and microemulsions.[97] Among the various approaches, microemulsion is most appealing due to its flexibility and expediency; monodisperse nanoparticles with defined morphologies and sizes may be produced. Moreover, limited aggregation may also be achieved. At a molecular level, surfactant molecules form solvent cages that control nucleation and subsequent growth, thus stabilizing water-in-oil microemulsion solutions.[98] The efficiency of these syntheses depends on several parameters, including calcium and phosphate ion concentrations, pH, ionic strength, temperature, and surfactant concentration and type.[99–101]

3.6. Silica nanoparticles

Silica nanoparticles may be prepared by sol–gel methods similar to those described previously. In particular, work by Stöeber et al. elucidated an efficient co-condensation process to afford monodisperse silica nanoparticles;[102] this work was later expanded through studies to covalently modify the silica surface and incorporate functional groups, including 3-aminopropylethoxysilane, *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane, 3-[2-(2-amino-ethylamino)ethylamino]propyltrimethoxysilane ureidopropyltrimethoxysilane, 3-isocyanato-propyltriethoxysilane, 3-cyanopropyltriethoxysilane, and allyltrimethoxysilane.[103–105] Additionally, microemulsion-based methods have been described to prepare silica nanoparticles, such as the organic–aqueous biphasic system described by the Tan group.[106] Solvent cages formed within the microemulsion direct the size and aggregation properties of the growing silica nanoparticles. As described previously, MCM-41 is a mesoporous silica nanoparticle. These nanoparticles are typically synthesized via similar sol–gel processes in the presence of a surfactant such as C_{12}-trimethylammonium bromide versus C_{16}-trimethylammonium bromide, to control pore sizes.[19] The relative ease of synthesis and functionalization make silica nanoparticles attractive targets for drug delivery; however the lack of information on their biodegradation remains a noteworthy limitation.

3.7. Fullerenes

Fullerenes are similar to carbon nanotubes in that their molecular framework is entirely composed of an extensive π-conjugated carbon skeleton. They are typically synthesized by poorly understood empirical methods; for instance, the vaporization of graphite by resistive heating yields grunge from which fullerenes can be isolated chromatographically.[107,108] Similarly, fullerenes can be synthesized in greater efficiencies after the combustion of simple hydrocarbons in fuel-rich flames.[109,110] These basic techniques provide abundant access to fullerenes; however, more elaborate

synthetic strategies are required for customized fullerene systems. The chemistry of geodesic polyarenes was investigated by the Scott group to prepare fullerene components, which were ultimately united via UV laser irradiation to yield C_{60}.[111,112] Four advances made this synthesis feasible: curvature was provisionally induced in polyarenes via flash-vacuum pyrolysis, radical-initiated C(aryl)–C(aryl) coupling reactions were designed to interdict the distorted conformations, facile 1,2-hydrogen shifts were exploited to limit challenging synthetic transformations, and cyclodehydrogenation cascades stitched the developing π-system together once curvature was induced.[113] Significant synthetic challenges remain, with future goals involving the preparation of higher order fullerenes, ^{13}C-labeled fullerenes, heterofullerenes, and azafullerenes.

3.8. Quantum dots

Quantum dots are luminescent nanoparticles typically used for imaging in biological systems. Their primary components—core, shell, and coating—have characteristics which each modify the photochemical properties. Quantum dots can be manufactured with diameters from a few nanometers to micrometers and a narrow size distribution using techniques requiring high annealing temperatures.[114] Bare core nanoparticles are labile as a result of their large surface area-to-volume ratio; they may also display emission irregularities resulting from surface imperfections.[114] Capping of quantum dots with ZnS has been shown to augment stability and enhance luminescence with superior quantum yields at room temperature.[115,116] However, ZnS capping alone is not sufficient to fully stabilize the core, especially in biological systems. PEGylation plays a dual role in increasing biocompatibility and improving the core stability in biological systems.[117]

4. Nanoparticle pharmacokinetics

4.1. Distribution

The natural clearance and excretion mechanisms of the human body provide a framework for the rational design of effective nanoparticles for use in medical therapies. Once a pharmaceutical agent is introduced into the circulatory system, for example by intravenous administration, it is distributed systemically via the vascular and lymphatic systems. The distribution of a drug in a tissue is correlated with the relative amount of cardiac output passing through that tissue. Accordingly, tissues and organs with high blood flow (brain, liver, heart, intestines, lungs, kidneys, spleen, etc.) may be exposed to higher concentrations of a drug, providing that the drug is able to penetrate into the particular tissue from the vasculature. A physiological parameter (cardiac output) can therefore act as a filter to nanomaterial distribution.

Another passive targeting mechanism involves altering the size of the nanoparticle carrier, which also alters the biological distribution profile. Very small nanomaterials, on the order of 1–20 nm, have long circulatory residence times and slower extravasation from the vasculature into interstitial spaces.[118] This may cause slower attainment of the maximal volume of distribution, or even an altered volume of distribution when administered intravenously. Local injections require an engineering of nanoparticles of slightly larger sizes, on the order of 30–100 nm. The latter size range is sufficient to avoid leakage into capillaries, but also small enough to avoid reticuloendothelial clearance (Fig. 3).[119,120] Moreover, surface manipulation can control the extent of localization at interstitial sites and limit clearance.

As nanomaterials are 'stealthed' via hydrophilic PEGylation, their circulatory residence times increase.[121,122] Thus, nanomaterials can become circulating depots of drug. The distribution properties of the drug ultimately depend on the kinetics of payload movement from the nanoparticle carrier; fast loss of the drug payload before the nanoparticle reaches its target may result in decreased drug efficacy. In a sphere, the ratio of the surface area to the volume ratio is inversely proportional to the radius.[123] As internally-loaded nanoparticles become smaller, a greater proportion of the drug payload will be located on the surface and have access to the exterior aqueous phase. This may lead to significant alterations in the pharmacokinetic parameters displayed by nanoparticles of various sizes and loading strategies.

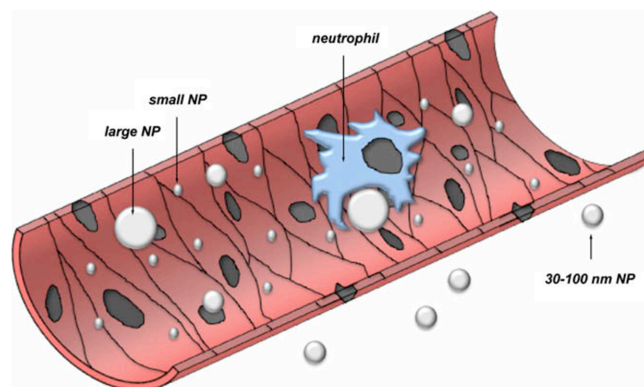

Figure 3. Very small nanoparticles, on the order of 1–20 nm, have long circulatory residence times with slow extravasation from the vasculature. Nanoparticles that are between 30 and 100 nm in diameter are small enough to avoid reticuloendothelial and phagocytic clearance, in contrast to larger nanoparticles, which are efficiently cleared.

Endothelial damage or alteration may modify the distribution parameters of nanoparticles. Inflammation, solid tumors, and deliberate disruption of endothelia contribute to an increased leakiness that provides vascular contents greater access to extravascular targets. Tumor growth triggers rapid angiogenesis, which results in extensive vascular networks with highly-fenestrated and 'leaky' endothelial cells; smooth muscle may incompletely surround these vessels, or may be absent.[124] Moreover, the blood-brain barrier may be weakened by solid tumors such as glioblastoma multiforme, thus providing better distribution of therapeutic agents to the CNS and tumor. Nanoparticles greater than approximately 100–150 nm in diameter will tend to accumulate in tumors due to their poor extravasation from normal vasculature.[123] The presence of disturbed, porous vascular beds at the tumor allows for selective targeting by this passive mechanism.

While passive targeting relies on a specific physiological parameter to act as a distributive filter, several examples of active targeting are known. The surface of nanomaterials can be ligated to a biological marker, such as an RGD peptide, an antibody, or an aptamer (Fig. 4).[91b,125–127] Nanomaterials present an unique opportunity to deliver pharmaceutical agents to tissues of interest. A firm understanding of specific cell markers, ligands, linkers, and the corresponding molecular biology is required for the future development of active targeting mechanisms. The nanostructure may be designed to encapsulate, or otherwise mask, the properties of the therapeutic agent. The delivery of the drug to a tissue whereby penetration and distribution may not otherwise occur is possible with these 'Trojan Horse' strategies. They have been utilized in a variety of medical settings, ranging from transport across the blood-brain barrier, targeting of drugs to tumor cells, and localization in vascular tissues.

4.2. Clearance/excretion

Whereas larger particles tend to remain localized, smaller particles are better able to clear from a placement site. For example,

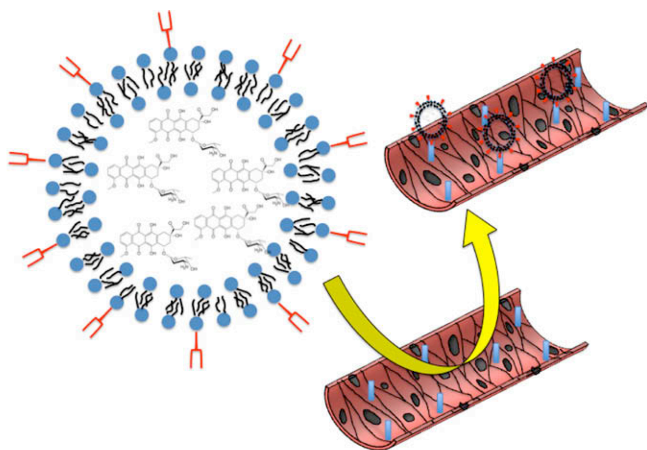

Figure 4. Active targeting of a doxorubicin payload within liposomes to the vascular endothelium using murine monoclonal antibodies to E-selectin, as reported by Spragg et al.[126]

60 μm polymeric microparticles composed of a slowly degrading polymer were locally injected at the sciatic nerve. Eight weeks later, the same microparticles were discovered in quantity at this injection site.[128] Similarly, microparticles (5, 25, 60, and 250 μm) injected into the peritoneum of mice remained there for at least two weeks. In contrast, nanoparticles of the same material showed almost complete clearance from the peritoneum in the same time frame.[129] Moreover, the spleens of mice treated with nanoparticles were enlarged and exhibited numerous foamy macrophages, presumably resulting from accumulation of a large amount of polymeric material. This finding obviates the role of the reticuloendothelial system in removing foreign objects—including nanomaterials—from the biological milieu. As part of the general immune response, monocytes and macrophages readily absorb circulating nanomaterials and then accumulate in lymph nodes and spleen for further processing.

Nanoparticles must therefore evade the reticuloendothelial system to be effective drug delivery agents. Many strategies for covert delivery may be implemented. As previously mentioned, PEGylation represents one approach to stealth nanomaterials. Hydrophobic nanoparticles such as unmodified liposomes are rapidly cleared via the reticuloendothelial system. The circulation times of these particles can be greatly increased simply by hydrophilic surface modification with PEG.[122]

Following systemic administration, the body normally distributes nutrients, clears waste, and distributes drugs via the vascular and lymphatic systems. Intravenously injected particles are scavenged and cleared from circulation by the reticuloendothelial system in a process that is facilitated by surface deposition of opsonic factors and complement proteins on the nanoparticles themselves.[130–132] Both clearance and opsonization are influenced by the size and surface characteristics of injected nanoparticles. Particles greater than 200 nm in diameter activate the complement system more efficiently and are cleared more rapidly than very small nanoparticles. This may be a result of the geometry, charge, and functional groups on the surface of these particles that mediate binding to proteins and blood opsonins.[133,134]

4.3. Toxicity

Many aspects of nanoparticle architecture and composition influence systemic toxicity. Care must be taken regarding the relative size difference between nanoparticles and the vasculature diameter. Particles >5 μm in diameter may embolize these vessels. Moreover, <100 nm particles have a high likelihood of aggregating; thus forming a cluster that can embolize and occlude blood flow. This property has been used to intentionally occlude the vasculature of tumors in the clinical setting, such as with the transarterial chemoembolization of hepatocellular carcinoma and other metastatic neuroendocrine tumors of the gastrointestinal tract. Alternatively, undesired consequences may also result, including lodging of these aggregates in various organs. For example, intravenous administration of nanoparticles prone to aggregation can result in a pulmonary embolism, strokes, myocardial infarctions, and other microinfarctions at distant sites and organs. Particles up to 4–5 μm in size could be injected directly into the carotid arteries of mice without producing detectable problems, with a caveat that very large quantities were not tested.[135] Thus, nanoparticle administration should result in no adverse embolic phenomena, providing the nanoparticles do not aggregate.

While the systemic toxicity profile of nanomaterials remains generally uncharacterized, a large body of information reports pulmonary and cardiovascular toxicities. There are striking parallels between nanomaterials and 'ultra-fine particles' in atmospheric pollution (nanoparticles traditionally defined as particles with diameters <100 nm, produced incidentally from industrial, combustion, welding, automobile, soil, diesel, and volcanic activities).[136] It has long been known that the lungs and cardiovascular system are particularly susceptible to inflammation and other pathologies following inhalation of these ultra-fine substances. For instance, silica-based particles have been shown to be promoters of inflammation and free radical damage with chronic exposure and in high doses; this phenomenon correlates with devastating pulmonary silicoses.

Epidemiological studies amongst six polluted and less-polluted American cities found a convincing association of ambient particulate air pollution as a predictor of mortality and morbidity in adults.[137–140] These studies concluded that exposure to ambient air pollution was associated with an increase in blood pressure and decrease heart rate variability. Furthermore, elevated levels of air pollution are associated with an increased incidence of asthma, life-threatening arrhythmias, and myocardial infarctions. While epidemiological studies do not necessarily define causality, they show a striking correlation with pollutant nanoparticles and adverse health outcomes.

Several mechanisms have been proposed to explain the correlations between nanoparticle inhalation and cardiopulmonary morbidity and mortality. First, neurons may be directly stimulated by nanoparticles, triggering alterations in the central nervous system and cardiovascular autonomic function. Additionally, the potential for neuronal uptake and translocation of inhaled nanoparticles to the brain has been reported in several studies. Roughly 40 years ago, De Lorenzo demonstrated in squirrel monkeys that intranasally administered colloidal gold nanoparticles (∼50 nm) translocated anterogradely in the axons of the olfactory nerves to the olfactory bulbs.[141] Using electron microscopy, the movements of these 50 nm gold nanoparticles were observed traversing synapses to the olfactory glomerulus within 1 h of intranasal administration, with a calculated neuronal transport velocity of 2.5 mm/h. Interestingly, and perhaps related to potential toxicities, it was found that nanoparticles in the olfactory bulb were not freely distributed in the cytoplasm; instead, they were preferentially located in mitochondria.[142a] A study of ^{13}C-labeled nanoparticles (∼36 nm) in rats confirmed these findings, demonstrating translocation via the nasal mucosa into the olfactory bulb.[142b] This pathway appears to circumvent the blood-brain barrier and may be exploited as a delivery alternative for drugs and nanoparticles that are otherwise unable to breach the blood-brain barrier. The precise details of this pathway are not currently known, including whether receptor-mediated endocytosis, pinocytosis, or axonal transport along cytoskeletal elements are involved. Moreover, it is not apparent whether these particles cause injury or toxicity to the brain, or

even if analogous pathways exist in other peripheral neurons. It is likely that inflammation of the olfactory mucosa, olfactory bulb, and cortical and subcortical regions of the brain may result. Consistent with the neuronal translocation mechanism, this inflammation was observed in dogs from a heavily-polluted area of Mexico City, but not in dogs from less-polluted areas.[143]

As described above, nanoparticles may trigger an inflammatory process resulting in the release of cytokines and chemokines, such as IL-6, (IL)-1β, TNF-α, reactive oxygen species, C-reactive protein, and transcription factors.[144,145] This cascade results in the activation of mitogen-activating protein kinase (MAPK), redox sensitive transcription factors, nuclear factor kappa B (NF-κB), and activating protein-1 (AP-1). By analogy, the etiology of atherosclerosis and coronary heart disease is thought to be inflammatory, as patients display similar pro-inflammatory markers.[146] These inflammatory mechanisms can lead to cardiopulmonary events. Studies using genetically susceptible mice exposed to long-term nanoparticle air pollution showed an acceleration of atherosclerosis and vascular inflammation.[147] We may be able to infer that these nanoparticles may promote, if not trigger, low-level systemic inflammation at distant organs and tissues, depending on nanoparticle access to the vasculature via penetration of small blood vessels and capillaries.

One mechanism whereby nanoparticles trigger inflammation involves the generation of reactive oxygen species (ROS). This is believed to be due to the greater surface area of nanoparticles, thus permitting more interactions with the biological environment and cellular components, and the myriad of transition metals often associated with the preparation of these materials. For instance, interactions between polystyrene nanoparticles and associated transition metals were reported to have a synergistic effect in ROS generation and subsequent inflammation.[148] In an associated study of four types of nanoparticles (carbon black, cobalt, nickel, and titanium dioxide), comparable free radical generation was also observed.[149] Through these studies, some types of nanoparticles were shown to be potent inducers of oxidative stress in macrophages by activating heme oxygenase-1 and depleting intracellular glutathione. Many transition metals promote free radical formation via Fenton-like chemical pathways. Additionally, mice exposed via inhalation to single-wall carbon nanotubes exhibited noteworthy pulmonary pathologic changes at small and high doses. Granulomatous lesions with persistent inflammation were seen within 90 days at doses of 3.3–16.6 mg/kg of body weight.[150] At doses of 10–40 g/mouse of single-wall carbon nanotubes with minimal impurities, mice displayed a vigorous inflammatory response with the onset of pulmonary fibrosis, decreased pulmonary function, and reduced bacterial clearance.[151] The generalizability of these pulmonary and cardiovascular toxicities to other systems remains unknown.

5. Mechanisms of cellular targeting

5.1. Nanoparticle uptake by tissues

A succession of several membrane layers provides an obstacle for therapeutic agents attempting to target intracellular structures. During this process, compound is lost due to ineffective partitioning across biological membranes. The extent of partition across a membrane is related directly to the polarity of a molecule; nonpolar or lipophilic molecules easily bypass this obstacle with greater membrane penetration, generally via diffusion. However, the situation is much more complicated, as a myriad of other cellular processes directly effect the intracellular concentrations and effectiveness of the therapeutic agent. Variable efficiencies of endocytosis mechanisms, intracellular trafficking, release of the therapeutic agent into the cytoplasm, diffusion and translocation of the therapeutic agent to its susceptible target, and partition into the nucleus or other organelles alter the actual activity of the therapeutic agent (Fig. 5). Nanoparticles present an interesting opportunity for eliminating much of this 'waste' due to masking of the therapeutic agent from its biological environment; this effectively limits the influence of a compound's physical properties on intracellular drug concentrations. Instead, the properties and surface characteristics of the nanoparticle play a greater role in compound delivery and resulting intracellular drug concentrations.

Nanoparticles may be ingested and 'sampled' by curious cells. Endocytosis encompasses the process of membrane manipulation to envelope and absorb materials and includes three subtypes: phagocytosis, pinocytosis, and receptor-mediated endocytosis. Phagocytosis involves the ingestion of materials up to 10 μm in diameter,[123] and can be accomplished by fairly few cell types of the reticuloendothelial system, such as macrophages, neutrophils, and dendritic cells. Pinocytosis is an uptake mechanism that can be conducted by virtually all cell types, and normally involves ingestion of sub-micron material and substances in solution. Larger microparticles provide selective access to phagocytic cells, while smaller nanoparticles provide access to virtually all cell types. This distinct capability of nanoparticles may be utilized for the delivery of therapeutic agents to a wide array of cellular types and targets.

5.2. Cellular phagocytosis/endocytosis

Receptor-mediated endocytosis affords the potential for even greater selectivity in cellular targeting. The cellular membrane is dotted with a myriad of receptors, which upon extracellular binding to their respective ligands (or to nanoparticles whose surface is functionalized with ligands), transduce a signal to the intracellular space. This signal can trigger a multitude of biochemical pathways; however it may also cause internalization of the ligand and its appended nanoparticle via endocytosis. Caveolin- and clathrin-coated pits provide an illustration of receptor-mediated endocytosis. Typically, clathrin coats generate a membrane indentation with a radius of curvature as small as approximately 50 nm,[152] and invaginate further upon binding of the ligand. Cross-linking of receptors via ligands attached to nanoparticles results in a more pronounced membrane crater with subsequent enfolding and reunification of the cellular membrane to form an endosome. It has been shown that nanoparticle sizes between 25 and 50 nm are a requisite for optimal endocytosis and intracellular localization.[153,154,48,155,156] Furthermore, selective active targeting of nano-

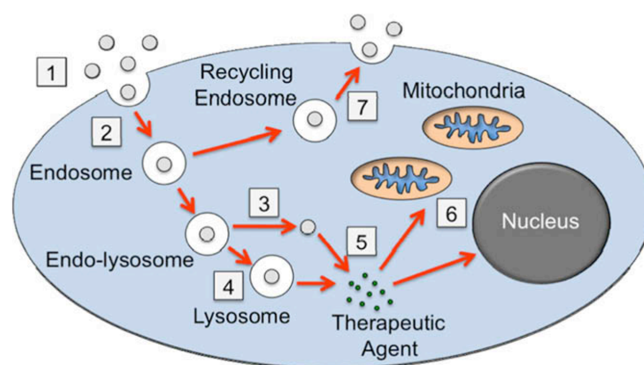

Figure 5. Steps detailing the cytosolic delivery of therapeutic agents via nanoparticle carriers. (1) Cellular association of nanoparticles, (2) internalization of nanoparticles via endocytosis, (3) endosomal escape of nanoparticles or (4) lysosomal degradation of nanoparticle, (5) therapeutic agent freely diffuses into cytoplasm, (6) cytoplasmic transport of therapeutic agent to target organelle, (7) exocytosis of nanoparticles.

particles to specific tissues may take advantage of the differential expression of receptors between cellular types. For example, it was recently reported that the attachment of multiple herceptin molecules onto the surface of nanoparticles prompted superior cross-linking of receptors overexpressed on human breast cancer cells such as ErbB2, with variable internalization depending on nanoparticle size.[156]

6. Nanoparticle drug delivery for human therapeutics

Nanoparticles have found widespread use in drug delivery, counting more than a dozen FDA-approved variants with indications ranging from cancer to infection (Table 1).

6.1. Neurological cancers (glioblastoma multiforme)

The central nervous system represents a formidable challenge for the delivery of therapeutic agents due to the blood-brain barrier (BBB). This physical barrier limits the brain uptake of the vast majority of neurotherapeutics and neuroimaging contrast agents. The anatomical and cellular morphology of neurovascular capillary endothelial cells, including limited pinocytosis and tight junctions, produces this unique central nervous system manifestation.[172] The brain microvasculature involves four types of cells: endothelial cells, pericytes, astrocyte foot processes, and nerve endings. Endothelial cells share the capillary basement membrane with pericytes that participate in immune surveillance. The other side of this basement membrane is almost entirely surrounded by astrocyte foot processes. Brain capillary endothelial cells are cemented together by tight junctions; this is associated with a 100-fold reduction of pinocytosis across the endothelium (Fig. 6).[173] Therefore, substances may gain access to the central nervous system by lipid-mediated free diffusion or potentially by receptor-mediated endocytosis of nanoparticles.

Nanotechnology may provide an effective means for circumventing this delivery issue past the BBB. Glioblastoma multiforme (GBM) is among the most devastating and lethal of neoplasms, often claiming the lives of patients within a median of one year following diagnosis. The treatment is multidisciplinary, including radiotherapy, chemotherapy, and surgery.[174] Transport of many chemotherapeutic agents past the BBB has proven difficult. Recently, however, a series of discoveries have been made. In particular, low-density lipoprotein receptors (LDLR) are upregulated on GBM cellular surfaces to between 128,000 and 950,000 receptors per tumor cell.[175] In contrast, average neurons have comparatively lower LDLR numbers, as evidenced by examining normal rat and monkey brain tissue.[176] Thus, targeting LDLR may offer the opportunity for potential therapeutic selectivity in chemotherapeutic drug delivery. Previous studies have investigated the use of low-density lipoproteins (LDL). Natural LDL particles are roughly 22–27 nm in diameter with a core of lipids primarily composed of cholesteryl esters with small amounts of triglyceride.[177] Initial studies used plasma-derived LDL as delivery agent to GBM tumors. However, due to the difficulty of isolating natural LDL, reconstituted and synthetic versions have become desirable.[178] Synthetic LDL nanoparticles have been shown to effectively deliver a toxic payload of paclitaxel to GBM tumor cells, and this cytotoxic effect is overturned upon treatment with the LDL receptor inhibitor suramin.[179]

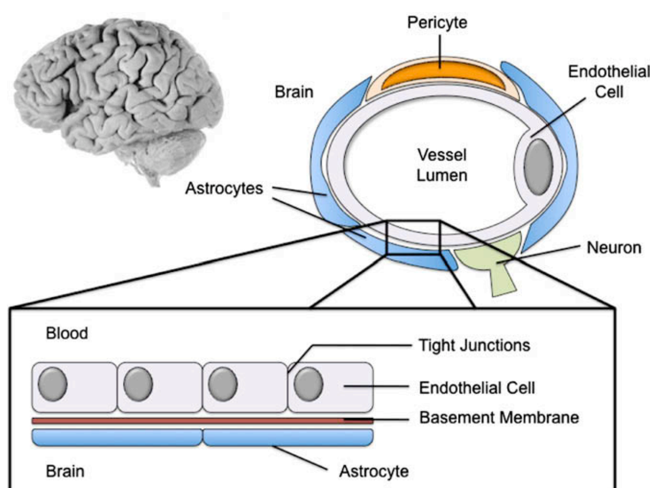

Figure 6. Anatomy of the blood-brain barrier (BBB).

6.2. Neurovascular diseases (vascular targeting and stroke)

Vascular diseases, such as atherosclerosis and hypertension, are a primary cause of neurological ischemia (strokes), aneurysm formation, and intracranial hemorrhage. Nanoparticles have been used diagnostically for the detection of atherosclerotic plaques; similar targeting strategies may be used to deliver therapeutic agents to these plaques. Early recognition and intervention may prevent dire neurological and systemic outcomes occurring subsequent to plaque rupture and subsequent thrombosis or embolism, as an example.

The natural course of atherosclerotic plaques is detailed in Figure 7. Atherosclerosis is primarily an inflammatory disease, with accumulating oxidized LDL particles triggering an inflammatory cascade with monocyte recruitment (Fig. 7A). Macrophages ingest these particles and are transformed into foam cells (Fig. 7B and C). The lipid core of the atherosclerotic plaque forms while

Table 1
FDA-approved nanoparticle drug delivery systems (adapted from Kingsley et al.) in clinical trials and/or use.[157]

Therapeutic agent (trade name)	Indication	Reference
Liposomal amphotericin B (Ambisome, Ablecet, Amphotec)	Fungal infections, Leishmaniasis	158
PEG-adenosine deaminase (Pegadamase)	Severe combined immunodeficiency disease	159
PEG-stabilized liposomal doxorubicin (Doxil, Evacet)	Kaposi's sarcoma, refractory ovarian cancer	160,161
Liposomal cytosine arabinoside (DepoCyt)	Lymphomatous meningitis, neoplastic meningitis	162,163
Interleukin 2-diptheria toxin fusion protein (Denileikin Diffitox)	Cutaneous T-cell lymphoma	164
Liposomal verteporfin (Visudyne)	Wet macular degeneration	165
PEG-interferon α-2b (Pegasys)	Hepatitis C	166
PEG-granulocyte colony stimulating factor (Neulasta)	Chemotherapy associated neutropenia	167
Protein bound paclitaxel (Abraxane)	Metastatic breast cancer	168
PEG L-asparaginase (Oncaspar)	Acute lymphocytic leukemia	169
PEG aptanib (Macugen)	Wet macular degeneration	53,170
Pemetrexed (Alimta)	Malignant pleural mesothelioma	171

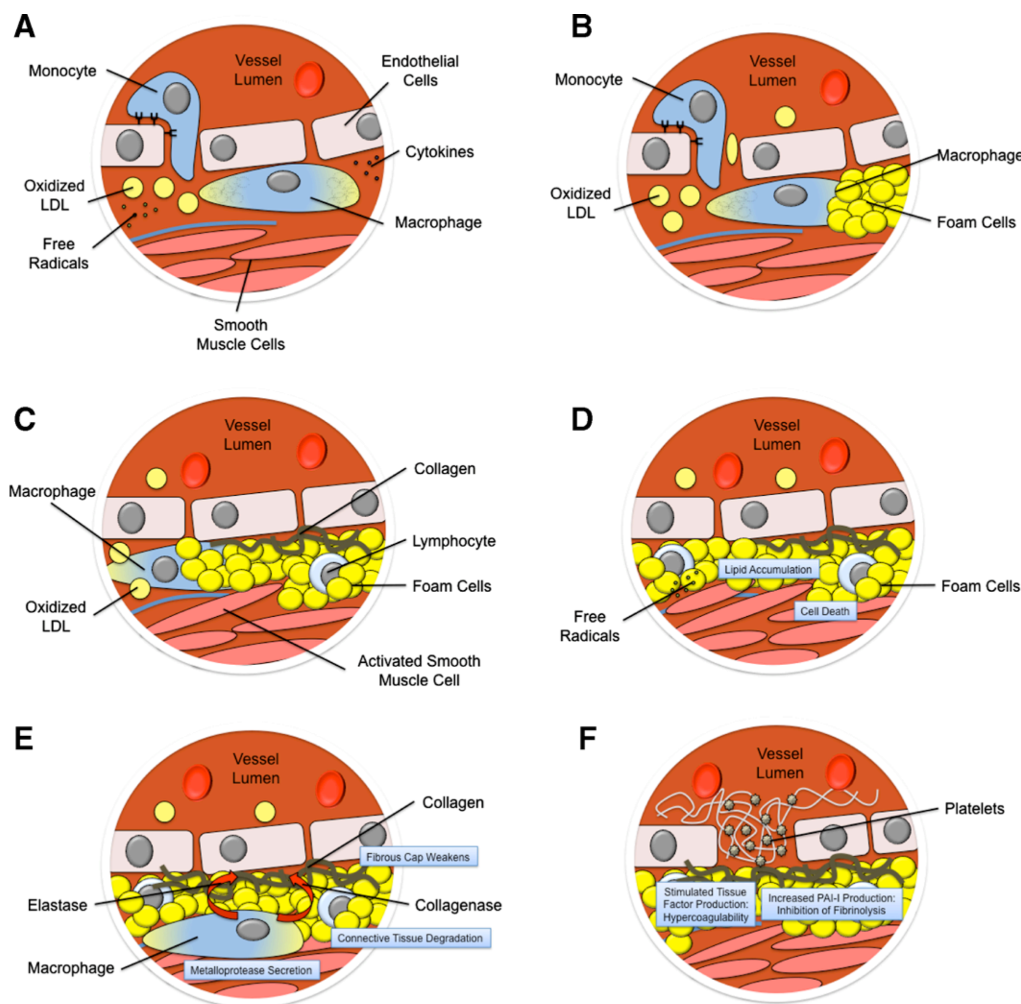

Figure 7. Natural course of atherosclerotic plaques.

smooth muscle cells in the blood vessel lining become activated and migrate inward (Fig. 7C and D). Simultaneously, collagen deposition occurs, strengthening the plaque and containing the subendothelial inflammation (Fig. 7C and D). Macrophages also liberate metalloproteases, elastases, and collagenases which degrade the connective tissue framework (Fig. 7E). Once this occurs, the plaque ruptures and prothrombotic factors, such as tissue factor, are accessible by circulating fibrinogen and platelets. Moreover, fibrinolysis is inhibited by increased production of plasminogen activator inhibitor-1 (PAI-1). Thus, a pseudo-stable clot forms with resumption of the inflammatory cycle (Fig. 7F). Rupture of an atherosclerotic plaque is a dangerous event—such as those occurring within the internal carotid arteries—with a high chance of embolus formation—this is a primary cause of neurological stroke and functional deficit in patients. Not only is fibrin deposition one of the earliest indicators of plaque rupture or erosion, but it forms a sizeable portion of the growing vascular lesion.[180]

Nanoparticle targeted fibrin imaging with ultrasound or paramagnetic magnetic resonance imaging contrast agents was initially demonstrated by the Lanza group more than 10 years ago.[181,182] In these examples, the targeting ligand consisted of an antibody fragment that was highly selective for cross-linked fibrin. This antibody fragment was conjugated to the nanoparticles via noncovalent avidin-biotin linkages or via direct covalent attachment.[182–184] As an extension, smooth muscle cells were harvested from porcine aorta and incubated with tissue factor-targeted nanoparticles loaded with paclitaxel. Specific binding of the nanoparticles elicited a substantial reduction in smooth muscle cell proliferation; non-targeted paclitaxel loaded nanoparticle administration resulted in normal proliferation.[184,185] More recent reports showed that intravenous administration of nanoparticles loaded with the antiangiogenic agent, fumagillin, targeted to $\alpha_V\beta_3$-integrin epitopes on the vasa vasorum of growing plaques resulted in a clear inhibition of angiogenesis in cholesterol-fed rabbits.[186] Kolodgie et al. utilized taxol-containing albumin nanoparticles to limit the restenotic response subsequent to angioplasty and stent placement in experimental animals.[187]

6.3. Neurodegenerative diseases (Alzheimer's disease and chelation)

Alzheimer's disease (AD) is marked by a progressive and irreversible damage to memory, thought, and language. It is extremely prevalent and represents the most common form of dementia in geriatric populations over 65 years of age. Current therapies include acetylcholinesterase inhibitors,[188,189] cholinesterase inhibitors,[190] antioxidants,[191,192] amyloid-β-targeted drugs, nerve growth factors,[193] γ-secretase inhibitors,[194,195] and vaccines against amyloid-β.[196] Mounting evidence suggests that oxidative stress triggered by various mechanisms may be a primary factor in neurodegeneration in AD.[191,197,198] Compared with other tissues, the central nervous system may be particularly susceptible

to oxidative stress, especially those catalyzed by transition metals such as iron and copper via Fenton chemistry.[199,200] In fact, iron metabolism has been shown to be involved in AD, as iron concentrations are elevated in patients with the disease.[201] Moreover, aluminum has also been found in high concentrations in senile plaques and intraneuronal neurofibrillary tangles within the brain of AD patients.[202] Unlike transition metals, aluminum is unable to participate in electron transfer reactions via redox cycling, as it assumes a fixed +3 oxidation state in biological systems. However, aluminum can act in synergy with iron to increase free radical damage.[202,203]

The promotion of oxidative damage by various metals in neurodegenerative diseases—such as AD—may represent a new target for drug design. In particular, chelation of these metals may reduce the pathophysiological development of AD. Metal chelators, such as desferrioxamine (DFO), have been used clinically.[204–206] DFO has strong affinities for iron, aluminum, copper, and zinc; the affinity constants for Fe(III), Al(III), Cu(II), and Zn(II) are 30.6, 22.0, 14.1, and 11.1 ($\log K$) respectively.[207] Unfortunately, DFO exhibits serious toxicity, including neurotoxicity and neurological changes;[208,209] it is poorly absorbed by the gastrointestinal tract and rapidly degrades following drug administration.[210] Penetration through the BBB may also be an issue due to DFO's hydrophilic nature, rendering it futile in neurodegenerative disease therapies.[211]

Polymeric nanoparticles may represent a potential means to transport drugs across the BBB.[212–214] Nanoparticles may be designed to mimic LDL and interact with the LDL receptor, consequently triggering uptake by brain endothelial cells. Nanoparticles may effectively mask covalently bound chelators, thus facilitating their delivery past the BBB and minimize toxicity while improving the pharmacokinetics of the chelator itself.

Seminal work by Liu et al. demonstrated the bidirectional transport of chelators into and from the brain.[215–217] These chelators were synthetically optimized and examined in brain tissue sections from AD patients. The synthetic chelators removed iron from ferritin more efficiently than DFO and were capable of removing iron from the brain tissue sections. Conjugation of these synthetic chelators to nanoparticles was achieved via covalent bonding to amino and carboxyl groups on the nanoparticle surface. To facilitate transport through the BBB via the LDL mechanism, the nanoparticles may be further functionalized with apolipoproteins. While there is much work to be done with these chelation-based nanoparticle systems, they exhibit potential utility for the treatment of AD and other neurodegenerative disorders.

7. Conclusions

Nanotechnology will assume an essential place in drug delivery and human therapeutics. A wide variety of nanoparticles exist already, and diverse methods of synthesis have been developed. The pharmacokinetic parameters of these nanoparticles may be altered according to size, shape, and surface functionalization. Careful design of nanoparticle delivery agents will result in successful localization and drug delivery to specific biological targets coupled with the efficient evasion of the reticuloendothelial system. Moreover, nanoparticles can be used to alter the kinetic profiles of drug release, leading to more sustained release of drugs with a reduced requirement for frequent dosing. Particularly interesting applications of nanoparticles in drug delivery relate to the central nervous system and the cardiovascular system. The blood-brain barrier is a formidable challenge for many therapeutic agents; nanotechnology may breach this barrier and establish a new frontier for neuropharmacologic agents.

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