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Chapter 9

Nano Delivery Systems

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Abbreviations

Ad	Adenovirus
BBB	blood brain barrier

BMV	brome mosaic virus	1
CCMV	cowpea chlorotic mottle virus	2
CPMV	cowpea mosaic virus	3
CNTs	carbon nanotubes	4
Cb	chlorambucil	5
CMC	critical micellar concentration	6
DDS	drug delivery system	7
EPR	enhanced permeability and retention	8
ELPs	elastin-like synthetic peptides	9
FHV	Flock House virus	10
AuNPs	Gold nanoparticles	1
HCRSV	Hibiscus chlorotic ringspot virus	2
IOs	iron oxide nanoparticles	3
Ir	Irinotecan	4
LCST	lower critical solution temperature	5
MRI	magnetic resonance imaging	6
MOFs	metal organic frameworks	7
MPS	mononuclear phagocyte system	8
MSNs	Mesoporous silica nanoparticles	9
NIR	near infra-red	20
NLNs	nanostructured lipid nanoparticles	1
HPMA	N-(2-hydroxypropyl) methacrylamide	2
PAMAM	polyamidoamine dendrimers	3
PVX	Potato virus X	4
PEG	polyethyleneglycol	5
PACA	poly (alkylcyanoacrylate)	6
PLA	poly (lactide)	7
PLGA	poly (lactide co-glycolide)	8
PCL	poly (e-caprolactone)	9
QDs	quantum dots	30
RCNMV	Red clover necrotic mottle virus	1
		2
		3
		4
		5
		36xy

RES	reticuloendothelial system
SLN	Solid lipid nanoparticles
SDDS	smart drug delivery system
NLSs	solid lipid nanoparticles
TEOS	tetraethoxysilane
TMV	Tobacco mosaic virus
TYMV	Turnip yellow mosaic virus
VNPs	Viral nanoparticles
VNPs	Viral nanoparticles
UV	ultra violet

I. Drug Delivery Systems

Delivery of a pharmaceutical compound through systemic circulation to the site of action to produce the desired therapeutic effect is the goal of drug delivery. In a biological system, numerous mechanisms exist to protect the body from exposure to foreign substances. A drug delivered in different ways will encounter physiological and biochemical barriers before reaching the site of action. Passage through these barriers depends on the physicochemical and biochemical properties of the drug molecule. Among these properties, solubility is very important for oral drug delivery. The lipophilic characteristics of the molecule are also essential for crossing cell membranes by diffusion. The molecule also has to survive biodegradation caused by the gastrointestinal system and the liver.[1] Figure 1 illustrates the importance of these physicochemical properties.[2]

The permeability (related to passive diffusion or a transporter-mediated process) and metabolic stability (related to intrinsic clearance) of a drug molecule are two important factors in drug delivery when the compound is in solution.

The goal of a drug delivery system is to provide enhance efficacy and reduced the toxicity of drug molecules. Long-circulating nanoparticles, such as liposomes, micelles, and polymeric nano-objects, can exploit the "enhanced permeability and retention" (EPR) effect for preferential extravasation from tumor vessels.[3]

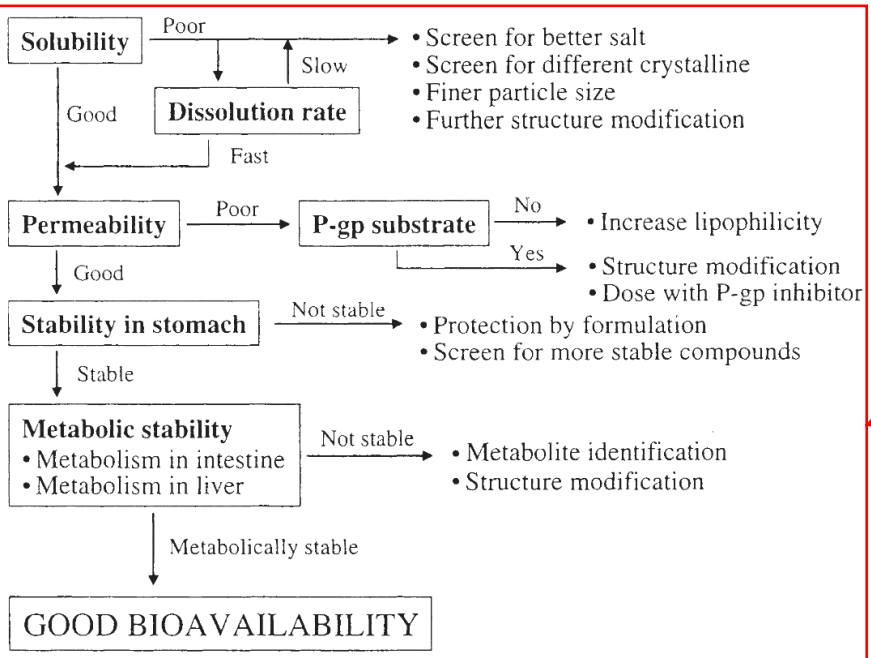

Figure 1. Importance of the physicochemical properties. (Taken from Ref [2] with permission of Wiley and Sons).

The currently approved stable formulations of liposomal drug delivery systems allow for improved pharmacokinetics and passive targeting to tumor tissues. Recent studies on drug carriers involve the direct molecular targeting of cancer cells via antibodies or other small ligands, such as peptides.

A. Nanoparticles in circulation: How to increase blood stability

Conventional liposomes are lipid vesicles essentially composed of various phospholipids (e.g. phosphatidylcholine, phosphatidylethanolamine). These systems suffer from low stability and rapid clearance from the blood.[4,5] Generally, they are rapidly taken up by macrophages in the liver. To overcome these problems, polymers can be used for

steric stabilization. Protecting the liposome surface with polyethyleneglycol (PEG) or other polar ligands, such as carbohydrates, allowed for the development of a stealth system.[6] PEGylation creates a hydrophilic and non-charged surface that can prevent early clearance of nanoparticles and increase their circulation time.[7] These long-circulating liposomes have improved pharmacokinetics compared to traditional systems. Surface coating prevents the nanosystems from agglomeration and from sticking to blood cells or vascular walls. They are invisible to the immune system and have shown promising results in cancer therapy. There is less uptake by the liver, and the liposomes can stay in circulation longer.

B. Nanoparticles for drug delivery

1. *Virus strategy*

To improve biodistribution and efficacy and to reduce the side effects of treatment, a number of nanostructures have been designed. Among them, the virus can be seen as a core/shell system consisting of an assembly of proteins enclosing genetic material. These nanostructures have naturally evolved to infect host cells with high efficiency and deliver their genetic material. Their size (20–300 nm) increases their chances of reaching the target cells, and some naturally differentiate healthy cells from tumor cells by preferentially targeting the latter. It is therefore possible to transport cargo diagnostics and therapeutic agents. For example, the functionalization of a capsid of a P22 bacteriophage with a contrast agent (Gd-DTPA) for magnetic resonance imaging (MRI) has improved the effectiveness by a factor of 6.[8]

Another interesting example is the ability of an alphavirus to transport molecules of interest preferentially in tumor cells. The advantage of viruses as nanocarriers[9] is that unlike other nanosystems, the number and orientation of the functional groups are well defined on the surface. Viral nanoparticles (VNPs) usually come from plants and bacteria. These particles are widely available and have a monodispersed structure. They have the advantage of being biocompatible and biodegradable and are considered to be non-infectious and safe

for humans. Their basic structure allows their cavity to be loaded with active molecules, and their surface can be functionalized with specific ligands.[10] It is also possible to generate VNPs by "genetic engineering" through chemical bio-conjugation or the self-assembly of proteins (Figure 2).

Virus, such as nanoparticles (VLPs), are generally produced from a baculovirus derived from insect cells or a mammalian adenovirus. They may also be produced from viral nanoparticles that are disassembled into protein subunits and reassembled once the nucleic acid has been removed. This type of particle is typically used in vaccinations.[10] The geometric shapes and sizes of VNPs and VLPs can be modulated by varying the pH, ionic strength, and physicochemical properties of the medium in which the protein is collected. The VNPs or their protein shell (VLPs) can also be used to encapsulate other types of particles,[11] such as nano-emulsions, NP polymers, enzymes, and inorganic NPs, forming more complex structures (Figure 3). Therefore, these nanoparticles are involved in many applications, such as gene delivery, catalysis, imaging, and the release of therapeutic agents.[10–14]

II. Chemical Engineering

Theranostic nanoparticles are multifunctional and are made from various building blocks of organic molecules (e.g. lipids, polymers, proteins, and polysaccharides) or inorganic components (e.g. iron, gold, metal oxides, carbon, and silica).

A. Drug conjugates

Drug conjugates are built from covalent or reversible interactions between two active ingredients or between a chemical cargo and an active substance. For example, the association between two anticancer drugs, such as Irinotecan (Ir) and Chlorambucil (Cb), via an ester bond allows the formation of an amphiphilic conjugate. The therapeutic efficacy of this conjugate for breast cancer is increased compared to the efficiency of each constituent taken separately (Figure 4).[15]

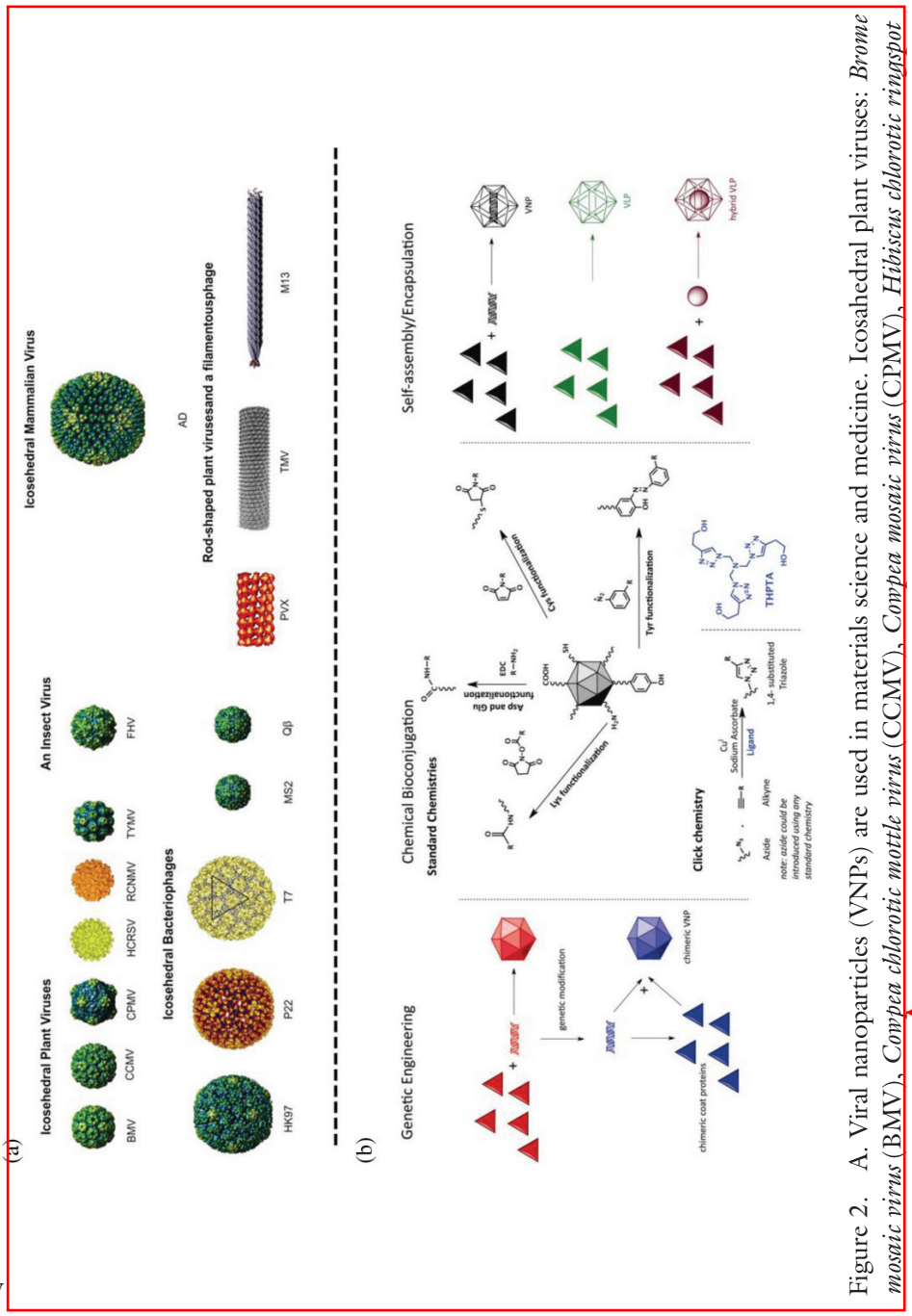

Figure 2. A. Viral nanoparticles (VNPs) are used in materials science and medicine. Icosahedral plant viruses: *Brome mosaic virus* (BMV), *Cowpea chlorotic mottle virus* (CCMV), *Cowpea mosaic virus* (CPMV), *Hibiscus chlorotic ringspot*

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Figure 2. (*Continued*) *virus* (HCRSV), *Red clover necrotic mottle virus* (RCNMV), and *Turnip yellow mosaic virus* (TYMV). Icosahedral insect virus: *Flock House virus* (FHV). Icosahedral bacteriophages: HK97, P22, T7, MS2 and Qβ. Note that P22 and T7 are head-tail phages with the tails not shown. Icosahedral mammalian virus: *Adenovirus* (Ad). Rod-shaped and filamentous viruses: *Potato virus X* (PVX), *Tobacco mosaic virus* (TMV), and bacteriophage M13. Images of the following VNPs were reproduced from the VIPER Database (http://www.viperdb.scripps.edu/): BMV, CCMV, CPMV, P22, TYMV, FHV, HK97, MS2, Ad, and Qβ. The structures of HCRSV, RCNMV, T7, PVX and TMV were reproduced from refs, respectively. B. Genetic, chemical, and self-assembly/ encapsulation manipulations of VNPs in biomedical research. (Taken from Ref. [10] with permission of Elsevier).

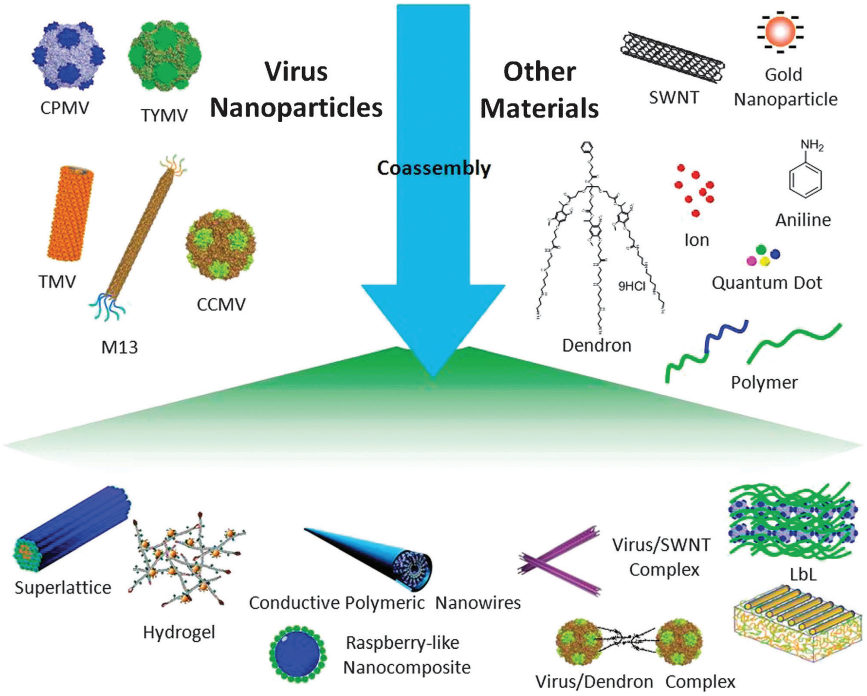

Figure 3. Self-assembly of hybridized VNPs with other materials. (Taken from Ref. [11] with permission of Royal Society of Chemistry).

For theranostics, two main classes of drug conjugates exploit the interactions between active substances (proteins, peptides or other active substances) and polymers.[16] For example, elastin-like synthetic peptides (ELPs) can be conjugated covalently to doxorubicin hydrazone cleavable linkages in an acid medium to increase their time of vascular remanence. The internalization of these prodrugs and their accumulation in lysozyme cleaves hydrazone bonds and releases active substances.[17] Albumin can also be covalently conjugated with anticancer drugs. For example, a methotrexate-albumin conjugate has been the subject of pre-clinical and clinical studies. The association is established by direct coupling between methotrexate and the lysine residues of albumin.[18] Synthetic polymers have also been developed as an alternative to previous polypeptide polymers. One polymer used

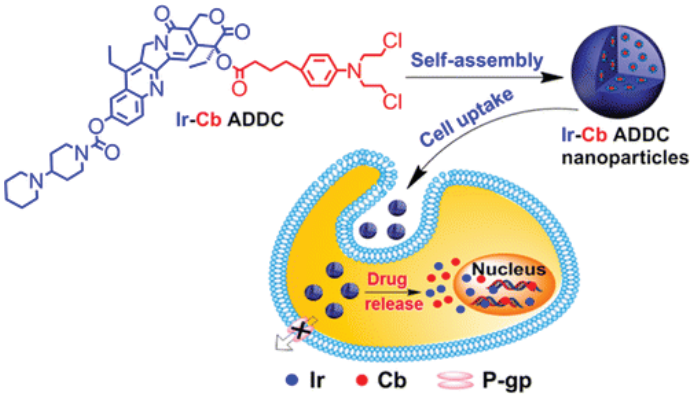

Figure 4. Example of an amphiphilic drug conjugate. (Taken from Ref. [15] with permission of American Chemical Society).

is N-(2-hydroxypropyl) methacrylamide (HPMA), which is known to be non-toxic, non-immunogenic and very stable. To date, several conjugated, HPMA-active substances are the subject of clinical trials, including HPMA-doxorubicin, HPMA-paclitaxel, and HPMA-platinates.[19, 20] These conjugated HPMA and active substances, such as anticancer and imaging agents, have been obtained by copolymerization[21] or chemical post-conjugation.[22]

B. Dendrimers

Dendrimers are highly branched synthetic polymers whose dimensions and physicochemical properties are close to those of biomolecules, such as proteins. They are produced by reactions in iterative steps; i.e., a tree.[23, 24] Both divergent and convergent approaches can be used. In the divergent approach,[25–27] the dendrimer is constructed from a heart that repeatedly emanates peripheral subunits. In this approach, the number of reaction sites is very important because it requires the use of highly selective reactions to avoid structural defects. In the convergent approach, the dendrimer is constructed by assembling dendrons.[28–30] In this approach, only a small number of reaction sites are activated at each step, thereby limiting the

number of side reactions per step. Therefore, polymerization is better controlled.

Due to their structure and multivalent characteristics, dendrimers can be used as good hosts for guest substrates. The host-guest interactions can occur in the internal cavities of the dendrimer structure landscape (endo-receptor) or on the surface of the dendrimer (exo-receptor). This leads to the encapsulation of guest substrates (Figure 5).[31]

Because of their many surface sites, dendrimers can also be used to graft covalently active substances on their periphery (Figure 6).[32, 33]

Therefore, dendrimers can accommodate molecules of interest to bind with or be conjugated to different active substances for mono- or multi-modal applications. For example, polyamidoamine dendrimers (PAMAM) conjugated to iron oxide nanoparticles have

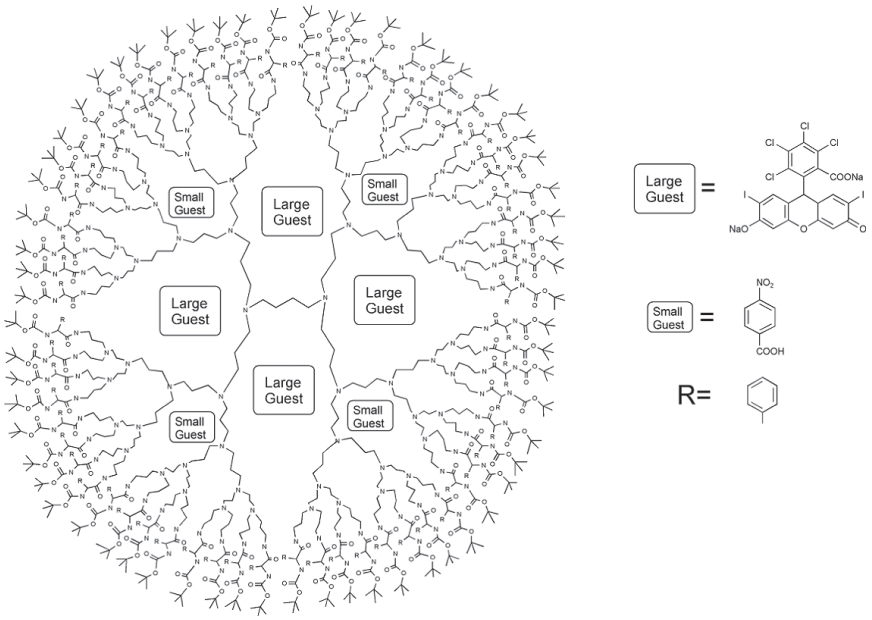

Figure 5. Representation of a dendritic box. (Taken from Ref. [31] with permission of American Chemical Society).

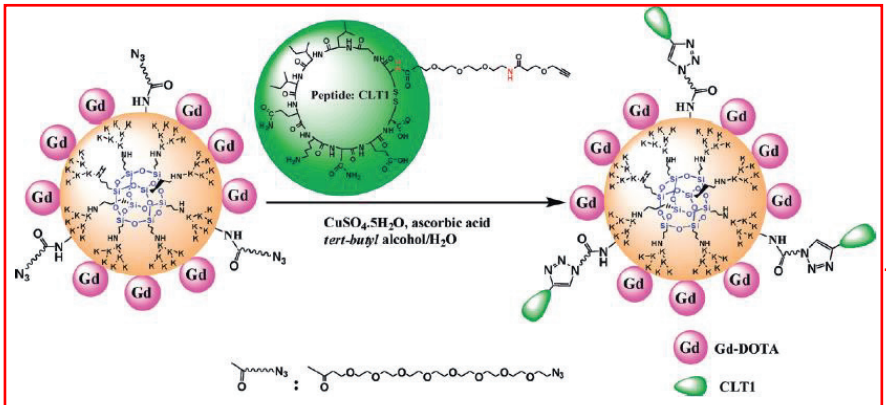

Figure 6. Dendrimer grafted periphery. (Taken from Ref. [33] with permission of American Chemical Society).

been studied as magneto-guidable transfection vectors of antisense oligodeoxynucleotides. Another more recent example is the encapsulation of a silicon naphthalocyanine within the hydrophobic cavity of fifth-generation polypropylenimine dendrimers. Depending on the light dose received and the power of the exciting laser, the naphthalocyanine can be used for optical imaging, PDT, PTT or all three simultaneously.[34]

C. Inorganic nanoparticles

Unlike polymer nanoparticles and dendrimers, which are primarily used as "carriers" of active substances, most inorganic nanoparticles have imaging agent properties and can present a therapeutic effect. For example, iron oxide nanoparticles can be used as contrast agent for MRI or therapeutic agents in the case of hyperthermia therapy.

Several types of inorganic nanoparticles (e.g. carbon nanotubes (CNTs), fullerenes, iron oxide nanoparticles (IOs), quantum dots (QDs), and gold nanoparticles) have been developed with a theranostic goal.[35,36]

D. Carbon nanotubes and fullerenes

Carbon nanotubes (CNTs) and fullerenes (C_{60}) comprise only carbon materials. Carbon nanotubes are made of graphite sheets wound on themselves. They may consist of a single layer of graphite (single-walled carbon nanotubes) or multiple layers (multi-walled carbon nanotubes) to improve their resistance.[37–39] The properties of the CNTs, and their optical properties in particular, depend on their size and the number of sheets that make up the tubes. The synthesis of nanotubes may be particularly oriented to impart good light absorption properties and a broad emission spectrum[39] for use in near infrared and Raman spectroscopy, for example.[40]

CNTs can also be functionalized with contrast agents for MRI and PET imaging applications.[41] For this, the surface of the CNT must be modified to develop graft sites by the introduction of surface defects (i.e. carboxylic acid).[42] For the non-covalent interaction of $\pi-\pi$ type and due to a large surface area, active substances can be associated with nanotubes.[43] Therefore, CNTs are credited with significant amounts of doxorubicin. As the $\pi-\pi$ interactions are pH dependent, the active compounds can be released by modulating the pH.[43] Fullerenes constitute a family of allotropic atoms in which the carbon atoms of the arrangement are composed of sheets of bonded hexagonal and pentagonal rings that prevent the sheet from being flat. Fullerenes have a hollow spherical structure of nanometric dimensions, which is useful to encapsulate active substances[44, 45] or contrast agents.[46–48] If the carbon nanotubes and fullerenes are interesting from a theranostic point of view, their *in vivo* toxicity is a crucial point to consider before clinical development.

E. Nanoscale iron oxide

Iron oxide nanoparticles are magnetic nanoparticles consisting of hematite or magnetite. They can be obtained by the co-precipitation of precursors of Fe (II) and Fe (III) in aqueous solution.[49,50] The aggregation of nanoparticles is avoided by covering the magnetic core with hydrophilic polymers, such as dextran or polyvinylpyrrolidone.

These polymers also provide functionalizable sites that make it possible to graft on the surface of the active substances or targeting systems.[50] Iron oxide nanoparticles can also be obtained by thermal decomposition of precursors of Fe (II) and Fe (III) in organic solvent.[51] This protocol allows better control of the size of the nanoparticles and their magnetic properties. However, because the nanoparticles that are obtained are hydrophobic, it is necessary to render them hydrophilic by ligand exchange or grafting to the surface.[52] Iron oxide nanoparticles that are 20 nm or smaller in size are characterized by superparamagnetic behavior.[53] They will affect the relaxation mechanism T_2 of the water molecules of protons and generate T_2-weighted images on MRI.[54, 55]

Iron oxide nanoparticles can also be used for the magnetic guiding of active substances.[56] In this case, the active compounds can be grafted onto the surface of the nanoparticles by amination reaction or co-encapsulated with iron oxides in polymer matrices.[54, 56]

F. Quantum dots

Quantum dots (QDs) are semiconductor nanocrystals based on CdTe, PbS or Cd_3P_2, CdTe/CdSe, InAs/ZnSe, and InAs/InP/ZnSe and are generally covered with ZnS.[57, 58] They behave like a potential well that confines the electrons (and holes) in the three dimensions of space in a region of order size of the wavelength of electrons (wavelength of de Broglie), which is a few tenths of a nanometer. QDs are characterized by a very narrow emission spectrum whose wavelength may be modulated by their composition and size. They are also extremely bright and more photostable than are organic fluorophores, which makes them interesting for biomedical applications.[58] QDs are obtained by heating organometallic precursors in a high boiling solvent in the presence of surfactants (trioctylphosphine or trioctylphosphine oxide) to control particle growth. The organic layer surface may be functionalized to make water-soluble QDs. QDs' affinity for the sulfur can be used for compounds such as mercaptosuccinic acid, glutathione or cysteine.[57,58] Due to the toxicity of the metals entering into the composition of the QD, few *in vivo* tests using QD

conjugates with active substances have been reported even if the QD may be functionalized. However, the use of theragnostic QD conjugated to doxorubucin and the RNA aptamer A10 for detecting a specific cell marker for prostate cancer has been reported.[59]

G. Gold nanoparticles

Gold nanoparticles (AuNPs) are most often obtained by reducing gold salts in the presence of a polar solvent and a surfactant. This acts as an agent to protect metal particles because when it is adsorbed on the particle surface, it prevents particles from agglomerating.[60,61] The morphology and nanoparticle size distribution are controlled by parameters such as reduction kinetics and the nature of the stabilizer. The shape of the AuNPs determines their optical properties, in particular by adjusting the absorption wavelength plasmon nanoparticles (the plasmon band for spherical AuNPs is approximately 500 nm and that for AuNPs formed of rods is between 650 and 900 nm).[62] In terms of the functionalization of AuNPs, the strong interaction of gold to sulfur can be advantageously exploited. The biomolecules can be thiolated and then grafted onto AuNPs.[60,61] Similarly, the AuNPs can be conjugated to antibodies. AuNPs[62] have strengths for use in theragnostics. These nanoparticles are not only stable, biocompatible and easily functionalizable but also have optical exploitable properties, including photoacoustics, photothermal ablation and SERS spectroscopy.[62] An example of AuNPs for theragnostics that is in clinical trials for the photothermal ablation of tumors is AuroLase® (Nanospectra Biosciences).[64] Finally, gold has a high atomic number, a high electron density and a strong attenuation coefficient of X-rays, making it a good contrast agent for X-ray tomography.[60] However AuNPs are non-biodegradable and their possible persistence can cause toxicity in the long term.[65] Even if their biocompatibility is proven, it is possible that the surfactant, reducing and other molecules used for their preparation can lead to inflammatory processes. Currently, the question of *in vivo* and *in vitro* toxicity of AuNPs is quite controversial and hampers their clinical use.[66]

H. Silica nanoparticles

Silica is a non-toxic, biocompatible material. Silica nanoparticles can be synthesized with good control of their size and shape. Their surface chemistry allows them to functionalize easily with contrast agents, active substances and targeting agents.[67] Silica nanoparticles are obtained by hydrolysis condensation of silicic precursors, such as tetraethoxysilane (TEOS).[68] The synthesis protocol may include co-precursors, such as aminopropyltrimethoxysilane or mercaptopropylmethoxysilane to introduce amine or thiol functions on the surface.[68] Substrates, such as antibodies, contrast agents, and fluorescent probes, can be introduced during synthesis provided that they have previously been coupled to one of the known co-precursors.[68] The nanoparticles may further be rendered mesoporous if a surfactant, such as n-alkyl trialkoxysilane, is introduced at the same time as TEOS in the synthesis.[69–71] Once the particles have formed, the surfactant is removed using a solvent, thereby creating pores with diameters that can be perfectly controlled. The mesoporous silica particles that are obtained have a high surface area that is suitable for functionalization. In addition, their biodegradability can be adjusted, making them of interest for theranostics.[72] However, the capture of these nanoparticles by the reticuloendothelial system can be a potential source of toxicity. Recent studies have suggested that silica nanoparticles are removed rapidly enough or eventually degrade so that their long-term toxicity can be considered low.[73] Finally, the biodistribution of the nanoparticles can be enhanced by PEGylation or by grafting specific biomarkers (Figure 7).[74]

In Figure 7, nanoparticles are functionalized sequentially in three areas: the silica structure with a contrast agent (ATTO647N), the mesopores with a PDT agent (PdTPP), and the surface with ligand targeting tumor cells (cRGD).

I. Coordination polymers

Coordination polymers or MOFs (metal organic frameworks), represent the last class of ordered porous materials.[75–79] One of their

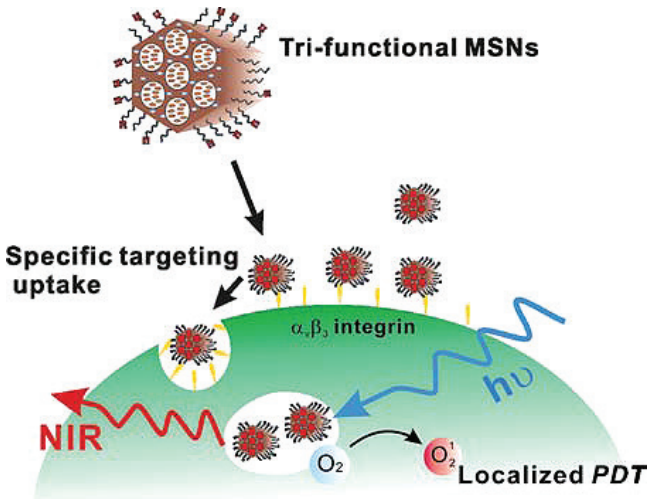

Figure 7. Mesoporous silica nanoparticles for theranostic applications. (Taken from Ref. [74] with permission of Royal society of chemistry).

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advantages over their organic counterparts, either carbonaceous or inorganic (zeolites, silica), is their ability to adjust their composition tailored by the choice of metal and/or organic constituent brick(s). Organic bricks are extremely varied (polycarboxylates, phosphonates, sulfonates, imidazolates, amines, pyridyl, and phenolates) and functionalizable.[80–82]

Functionalization can also be carried out retroactively on MOFs that are already formed.[80–82] In addition to their great chemical diversity, MOFs are characterized by a great diversity of shapes and pore sizes and there is the possibility of adjusting the pore size reversibly adsorbed to the substrate.[83,84] Therefore, one of their applications is their use as biomedical nano-cargos and systems for the controlled delivery of active substances.[83, 84] The use of these porous solids in biomedical applications requires the use of biocompatible components for the development of MOFs. The most suitable metals are those whose toxicity is low, such as Ca, Mg, Zn, Fe, Ti, and Zr. The most common building blocks are synthetic from natural compounds that will be inert with respect to biological cycles *in vivo* (e.g. 2, 5 dihydroxoterephthalate,[85] polycarboxylates,[86] adeninate-4,4–biphenyldicarboxylate).[87]

A second option is to use endogenous constituent bricks, such as fumarate, muconate,[88–90] cyclodextrins,[91] or amino acids.[92] Currently, the number of sufficiently porous framewords and/or MOFs that are suitable for theragnostic applications is small, but the development of new synthetic protocols in the near future should advance this field. Finally, it is possible to obtain nanoparticles from these porous materials. Control of the particle size is a key point because it determines the mechanical properties of these nanoMOFs (NMOFs). Obtaining a stable, homogenous nanoparticle monodisperse population in this area is also crucial. The hydrothermal method,[93,94] which involves the use of microemulsions in the reverse phase,[95–98] the sonochemical path,[99–102] and the path hydro/solvothermal assisted by microwaves,[103,104] allows access. The issue of biodegradability (inherent in the use of inorganic ions) can be addressed through the use of endogenous metals with a control concentration. In general, the choice of components is infinite, making it is possible to select ligands and metals that degrade or eliminate. Therefore, the compounds of iron carboxylate NMOFs are biocompatible and biodegradable and are an example of MOFs compatible with intravenous administration. In addition, their high load capacity and extended release make these NMOFs very attractive nano-carriers.[77]

J. Micelles and polymersomes

Micelles and polymersomes result from the self-assembly of surfactants or amphiphilic copolymers in solution. In an aqueous solution, the hydrophobic blocks of the amphiphilic molecules orient themselves to minimize adverse interactions with the aqueous environment. This organization takes place only above a certain concentration, called the critical micellar concentration (CMC).[105] The CMC depends on the chemical nature of the amphiphilic molecules. Generally, the higher the molecular weight of the hydrophobic portion, the lower the CMC.[105] As long as the amphiphilic concentration remains above the CMC, the assembly remains thermodynamically stable, but below the CMC, it disassembles with a specific speed that depends on the structure of and interactions between the chains. According to the physicochemical properties of the amphiphile,

aggregates formed can be of different sizes and shapes. Therefore, one can find aggregates formed from a single spherical or cylindrical layer (micelles simple type), two flat layers (lamellar), or closed as concentric hollow spheres. In the latter case, this is called vesicle architecture.[106–108] The vesicles, as opposed to micelles, are composed of at least two layers of amphiphilic derivatives. The vesicles may be constituted by a single lamina (uni-lamellar) or two or more sipes (multilamellar).

The micelles may be obtained using either common surfactants, such as Cremophor® and polysorbates (non-polymeric micelles), or amphiphilic copolymers (polymeric micelles).[108] The amphiphilic copolymers compared to low molecular weight surfactants have a lower CMC, and micelles can be more resistant to disassembly under the effect of dilution (particularly in the blood).[109–111] Therefore, polymeric micelles are preferable to non-polymeric micelles. The micelles have a heart-ring architecture in which the heart is composed of the hydrophobic portion, creating a space into which lipophilic molecules of interest may be solubilized. The ring formed by the hydrophilic part of the amphiphile generates a hindered opsonin and therefore makes it possible to increase the half-life in the bloodstream significantly. In addition, this ring may be functionalized by grafting hydrophilic markers or diagnostic agents (Figure 8).[112, 113]

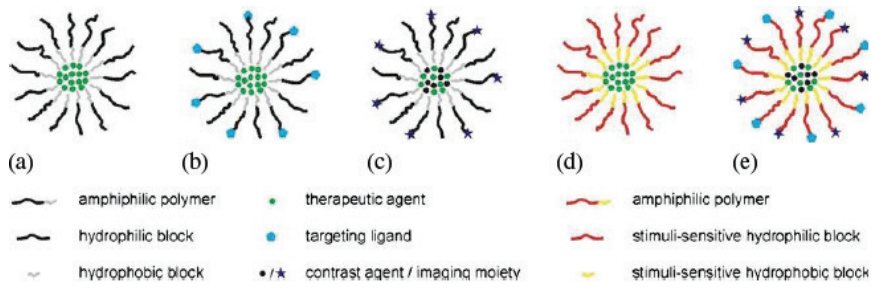

Figure 8. Schematic micelle polymer (a); functionalized micelle with targeting markers (b); theragnostic micelle (c); modified micelle for controlled release (d); and optimized micelle to treat cancer or thernagnostic micelle (e). (Taken from Ref. [107] with permission of Royal Society of Chemistry).

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Micelles can be obtained in different ways, including direct dissolution, dialysis, oil in water emulsion (O/W), evaporation of solvent, salting-out, or rehydrating a polymer film.[105] These methods can lead to differences in structure and load levels of active ingredients.[114] Their small size (typically <100 nm) reduces their elimination by phagocytes of the liver and spleen and maximizes the EPR effect. At the same time, the size is sufficient (>10 nm) to prevent renal excretion. Using polymers sensitive to temperature changes, pH, light or magnetic field controls the disassembly of the micelles and therefore the release of the active principle. Grafting recognition systems facilitate the preferential accumulation in the area of interest.[112,115–117] These features make micelles prime candidates to convey anticancer drugs or to serve as theranostic agents.[118–121] For example micelles used in theranostics, such as micelle polymers in which the amphiphilic unit carries a fluorescent group, have been developed and used for the delivery of doxorubicin. Modifying fluorescence micelles, which are controlled by their assembly and disassembly, can be used to track the release of active principle by fluorescence.

Moreover, the encapsulation of doxorubicin in these micelles enhances its antitumor activity *in vitro*.[120] The polymersomes are vesicles synthesized from amphiphilic copolymers of blocks that are organized as a sphere hollow (Figure 9). Two methods are primarily used: solvent change and rehydration of a polymer film.[122,123] In these hollow structures, the aqueous heart is surrounded by a bilayer wall. This membrane displays a hydrophilic interface on both the inner and outer surfaces. Between the two is the hydrophobic portion of the wall.[122] This organization can encapsulate hydrophilic molecules in the cavity and transport hydrophobic molecules and/or amphiphilics in the wall.

With this hydrophilic/hydrophobic duality, the ability to incorporate different molecules, and the robustness of the wall, polymersomes are smart polymers (Figure 10) to activate the release of active substances by polymersomes and their external functionalization may ensure better targeting.[125,126]

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Figure 9. Schematic representation of polymersome with a bilayer wall. The hydrophilic crown is shown in blue and the hydrophobic portion is shown in red. (Taken from Ref. [122] with permission of Elsevier).

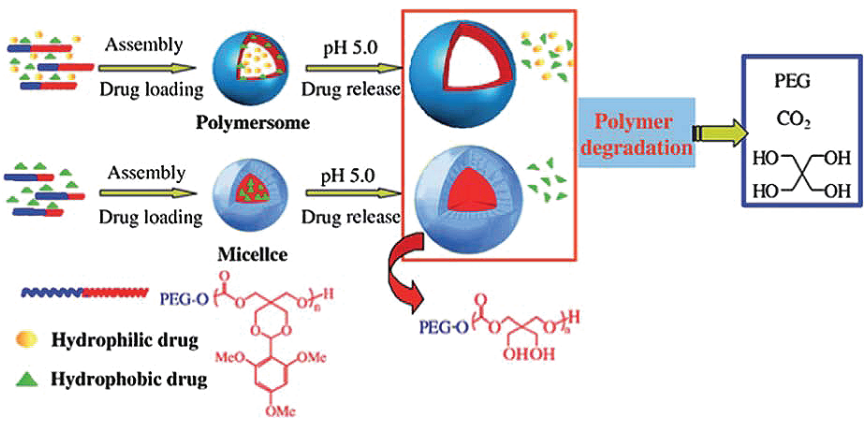

Figure 10. Smart polymersomes and micelles prepared with diblock that are PEG-sensitive to pH and PTMBPEC degradable. (Taken from Ref. [123] with permission of Royal society of chemistry).

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K. Liposomes and lipid nanoparticles

Liposomes are the result of the self-assembly of natural or synthetic lipids (cholesterol, phospholipids and derivatives) in water. They have a structure similar to that of polymersomes. These are spherical

vesicles with a hydrophilic cavity protected by one or more membrane bilayers whose polar heads oriented toward the outer surface and the internal volume.[127] Differences between liposomes and polymersomes come from the nature of the constituents. In the case of polymersomes, the polymers have a molar mass greater than that of the phospholipids used for the liposomes, resulting in the formation of a thicker membrane that is stiffer, stronger and less permeable.[111,128] The relative permeability of liposomes can result in premature or uncontrolled release. This weakness can be overcome by incorporating a liposome in other liposomes, similar to a nesting doll. These new structures, called vesosomes, have multiple membranes and allow for a delayed release of the molecule or molecules of interest.[128]

In vesosomes, internal compartments are separated from each other and may have different lipid compositions and encapsulate various substances.[129] Liposomes are classified according to the number of lipid layers (uni- or multilamellar) and size (small, large, and giant).[128,130,131] The size and number of the bilayers affect traffic, the half-life, and the charge rate of encapsulated molecules.[127] Multilamellar liposome sizes are obtained from the hydration of a lipid film. To form smaller unilamellar vesicles, it is necessary to use ultrasound or extrusion. A method involving dispersion solvent and detergent removal may also be employed.[127] Because of their biocompatibility, biodegradability, low toxicity and ability to transport all hydrophilic molecules, both lipophilic and amphiphilic, they are extensively used in various fields, such as cosmetics, pharmaceuticals, and food.[127,130–132]

Liposomes, which were discovered by Bangham in 1965, were cleared by the FDA for the first time for therapy in 1995 as Doxil® (Ben Venue Laboratory).[133] Since then, liposomes have been approved for clinical applications in oncology (DaunoXome® (Novex Pharma) and Myocet® (Cephalon)); virology (Inflexal V® (Crucell)); and ophthalmology (treatment of AMD Visudyne® (Novartis Pharma)).[133–136]

Most of these liposomes are cleared for intravenous and intramuscular administration (in the case of antivirals, such as Epaxal® and Inflexal®). However, the oral route is avoided due to the instability of liposomes in gastric fluids.[133,134]

These systems are also suitable for the encapsulation of diagnostic agents.[137,138] However, few studies have described the combination of therapeutic and diagnostic agents into liposomes.[136] The liposomes and lipid nanoemulsions are composed of excipients that are well tolerated *in vivo*, have a structure and composition similar to those of physiological membranes and oral bioavailability, and can be easily mass produced. However, they suffer from too much permeability and chemical sensitivity to oxidation and degradation compared to other nano-formulations.[131,132] In the mid-1990s, solid lipid nanoparticles (NLSs) were needed as an alternative to existing lipid nanoformulations.[139] NLSs combine the advantages of solid nanoparticles (stability, controlled release) with those of liposomes and nanoparticles liquid lipids (biocompatibility and good tolerance for many routes of administration).[139,140] NLSs are derived from oil-in-water (O/W) emulsion nanoparticles in which the liquid lipid is replaced by a solid lipid at room temperature and up to 37°C. The lipids used are physiological or recognized as biocompatible *in vivo*.[141] NLSs are prepared from lipids, surfactants, organic solvents and an aqueous phase using the one of many possible synthesis methods, including inversion temperature (PIT), high-pressure homogenization, dispersion of a film by ultrasonic emulsion-solvent evaporation, microemulsion, and the use of a supercritical fluid. The hydrophobic lipid cores of nanoparticles allow the encapsulation of therapeutic agents and diagnostic lipophilics.[139–142] The encapsulation of hydrophilic molecules, such as certain proteins or anticancer or contrast agents, for MRI is still possible. For example, the encapsulation of Gd-DOTA in an NLS produced by a double water-oil-water (W/O/W) emulsion was achieved.[143] Solid lipid nanoparticles have potential as theranostic agents.[144] An NLS has both advantages and a number of disadvantages, such as problematic reproducibility in the growth of nanoparticles and the possibility of polymorphic transitions that can induce the expulsion of the active substance during storage and lead to a low expense ratio.[139,140,145] These disadvantages can be overcome with second-generation lipid particles of a controlled nanostructure, which are called nanostructured lipid nanoparticles (NLNs). NLNs consist of a mixture of lipid solids and liquids that generate a matrix that

remains solid at 37°C, but the melting point decreases compared to an NLS. The solubility of the active ingredient increases.[145] These structures are very stable and have a high capacity for lipophilic agent loading. However, the incorporation of hydrophilic molecules is possible only in limited quantities.[146]

L. Polymeric nanoparticles

Polymeric nanoparticles are defined as colloidal particles prepared from polymers that range in size from 10 to 1000 nm. There are two types of nanosystems: nanocapsules and nanospheres.[147]

Nanocapsules can be defined as reservoir vesicles consisting of a liquid or semi-liquid heart (water or oil) surrounded by a solid polymer shell. The molecules to be conveyed can be encapsulated into the heart or be adsorbed to the surface of the nanocapsules. The nanospheres are matrix particles that consist of a completely solid polymer entanglement. The molecules of interest can be adsorbed to the surface or be dispersed throughout the matrix network of the nanospheres (Figure 13).[147–151] These nanoparticles are generally spherical. The structure of the nanospheres/nanocapsules and the adsorption mode of incorporation/encapsulation are driven by the choice of synthesis method and the nature of the molecules to be conveyed, respectively.[150, 151]

Fragile molecules, which are desired to preserve integrity *in vivo*, will preferably be encapsulated within the nanoparticle rather than adsorbed on its surface. By contrast, molecules that may lose their integrity at the time of encapsulation will be preferentially adsorbed after the synthesis of nanoparticles.[152–155] Polymeric nanoparticles have applications in many fields, including electronics, medicine, cosmetics, and technology.[147]

M. Polymeric nanoparticle design

Among all nano-objects of therapeutic and diagnostic interest, polymeric nanoparticles are currently the most studied for their numerous advantages, including biocompatibility, biodegradability, and chemical

versatility for the alteration of their physicochemical properties. The development of these nanocarriers has evolved from a simple structure to complex and carefully crafted assemblies.

III. Different Generations of Nanocarriers

Currently, there are four successive stages in the development of nanocarriers. They are listed in order of increasing responsiveness to the body and cancer pathology.

A. First-generation passive addressing

The first-generation polymer nanocarriers appeared shortly after the reported use of raw liposomes. These nanoparticles were "naked," with an unmodified surface of nanosphere or nanocapsule types (Figure 13). The active ingredients are dispersed in the molecular state in the matrix (nanospheres) or in an oily or aqueous heart (nanocaspules). A proportion of the active ingredients is adsorbed on the surface of the vectors. The most common polymers used for the synthesis of nanocargos are acrylates, such as poly (alkylcyanoacrylate) (PACA); polyesters, such as poly (lactide) (PLA), poly (lactide coglycolide) (PLGA), and poly (e-caprolactone) (PCL); and polysaccharides, such as chitosan, gelatin, and hyaluronic acid. All of these polymers share the properties of being biocompatible and biodegradable, which is caused by hydrolysis *in vivo* from acids or sugars naturally present in the body.

The first attempts to intravenously administer polymeric carriers resulted in rather disappointing results because a very rapid clearance (on the order of minutes) and limited biodistribustion, mainly in liver and spleen, were observed.[156] Adsorption of plasma proteins on the surface polymer nanocarriers, or opsonins, has been recognized as a factor responsible for the recognition of such particles by the reticuloendothelial system (RES). Hydrophobic interactions that develop these opsonins for polymer surfaces can be reduced by changing the nature of the polymer matrix. Unfortunately, these interactions cannot be prevented completely. Some therapeutic strategies,[157] however,

address this situation by targeting cancerous diseases of the liver. Therefore, Verdun *et al.*,[158] used nanoparticles (hexylcyanoacrylate) loaded with doxorubicin to treat diseases of the liver and spleen. In addition, the accumulation of nanoparticles in the heart and other organs reduced the cardiotoxicity of doxorubicin, which is a significant advance in their use.

B. Second-generation stealth

Because the first-generation nanoparticles (lipid or polymer) had an extremely short half-life, solutions based on the work initiated by the team of De Gennes on a "protein interaction surface" in the presence of poly (ethylene oxide) (PEO or PEG) were proposed.[159] These studies reported that the incompressibility of PEG chains grafted onto the surface of a hydrophobic solid repelled proteins and did not allow their adsorption onto the hydrophobic surface. This observation on the role of steric hindrance on the surface of nonionic polymers quickly led to the development of second-generation nanocarriers, which have a hydrophilic PEG crown surface. Because the adsorption of serum proteins on the surface of the nanoparticles is the leading cause of immune recognition and rapid clearance of nanocarriers, the plasma residence time increases significantly in the presence of a PEG surface of nanocarriers.[160] Therefore, these nanoparticles are called "stealth" to distinguish them from those of the first generation. This feature of stealth also alters the biodistribution of second-generation nanocarriers compared to those of the first generation. Because they have a longer circulating in the body, these nanoparticles can accumulate in tissues whose blood supply is looser for pathological reasons. This is the case in inflammatory tumor areas. Under the impulse of tumor growth, neovascularization is established quickly. It is characterized by an abnormal structure, disorganized and a more permeable vascular endothelium with fenestrations (pores) between the endothelial cells ranging from 300 to 500 nm. In this context, nanocarriers that are less than 200 nm may passively reach the tumor tissues. In addition, malfunction of the lymphatic drainage of the tumor areas slows down or even prevents the clearance of these

nanocarriers. This is the EPR effect (enhanced permeability and retention, Figure 11).[161] The efficiency of this approach, which was first demonstrated by Matsumura and Maeda,[162] on the accumulation of macromolecules in tumor tissue has been confirmed for various nanocarriers, including polymeric nanoparticles.[163]

The surface PEGylation of the nanoparticles can occur in different ways. The PEG may be covalently grafted to the hydrophobic polymer, or it may be co-precipitated during synthesis or adsorbed onto the surface. From these various steps, surface coatings with densities and various architectures will result. The optimum parameters for better stealth and biodistribution are adequate and not controversial at this time.[165] However, the accumulation of data on the use of PEG has highlighted a number of negatives attributes, including the fact that PEG interferes with cellular internalization and the endosomal escape phenomenon and could cause undue immune reactions.[165] For this reason, other surface-coating polymers have been studied and used successfully, in particular poloxamers, poloxamines and polysosides.[166] Regardless, PEG remains the ultimate stealth material, and numerous ongoing clinical trials are using PEGylated nanoparticles. For example, Abraxane® (Celgene) is an injectable, specialty-based

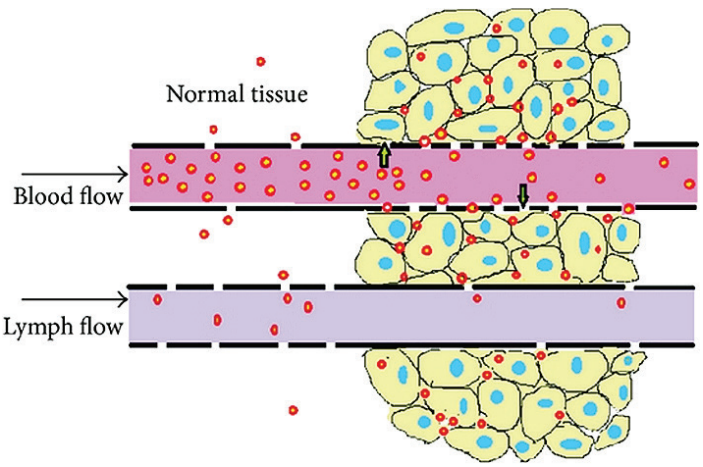

Figure 11. Schematic representation of the EPR effect. (Taken from Ref. [164] with permission of Hindawi).

paclitaxel (cleared for marketing since 2005) that is prescribed for the treatment of breast, lung and pancreatic cancer. These albumin nanoparticles carry the antineoplastic agent, and this was the first specialty polymer to obtain clearance to be placed on the market. Although it is devoid of PEG, this formulation may be regarded as a second-generation nanocarrier because albumin naturally attracts the RES, and the half-life of the nanoparticles is extended.[167]

C. Third generation: active targeting

The concept of active targeting is defined in opposition to that of passive targeting in tumor areas, or the EPR effect. The term "active targeting" refers to second-generation nanocarriers with surface specific ligands that target specific membrane cell receptors.[168]

Two targeting strategies can be employed. One strategy targets the tumor environment (e.g. endothelial cells) to deliver anti-angiogenic molecules or molecules that stimulate antitumor immunity. The other strategy targets cell membrane receptors of tumor cells for the intracellular delivery of antineoplastic molecules.[169] In the case of solid tumors, for active targeting to work properly, the accumulation of nanocarriers in the tumor areas (the EPR effect) is a prerequisite. Molecular recognition between the ligand and target membrane site can then take place, which triggers endocytosis of the nanocarrier and the release of molecules of interest.[170] The targeting entities to the surface of the nanocarrier can be very diverse and include, for example, monoclonal antibodies, lectin, aptamer, folate, and peptides.[171–173] Selecting this entity will, of course, be dictated by the tumor to be treated, and, in particular, the level of affinity between the ligand and its receptor.[174] Therefore, a candidate must have a high affinity for a specific receptor of tumor cells. It is also required that this receptor be present in large amounts on the surface of the target cell as well as on the ligand to the surface of the nanocarrier. This is one reason why the design and development of these vectors is so delicate and is still in the experimental stage. This strategic approach has nevertheless been proven in vivo. Farokhzad *et al.*,[173] have demonstrated the effectiveness of PLGA-PEG nanoparticles loaded with docetaxel on

human tumors implanted in nude mice. The presence of an aptamer that recognizes the extracellular domain of the antigen specific for prostate tumor cells (PSMAs) on the surface of the nanoparticles resulted in the complete regression of tumors, while nanocarriers that had had no aptamers slowed tumor growth.

However, despite these encouraging results, a major problem remains: the low penetration of nanocarriers in tumor areas, which is limited to a few millimeters around the blood vessels.[175] Therefore, the hearts of solid tumors can be reached by antineoplastic molecules, which do not eradicate the tumor.

D. Fourth-generation "smart" vectors

The term "intelligent vectors" refer to nanoparticles that are capable of responding to exogenous (e.g. magnetic or electric field, temperature rise) or endogenous (e.g. change in pH, change in concentration of an endogenous molecule, presence of an enzyme special) stimuli.[176] In response to the specific stimulus, the polymer matrix undergoes protonation hydrolytic cleavage or a conformational change that activates the release of the active substance.[177] Therefore, these nanocarriers enable the release and/or activation of molecules transported in a controlled manner. It is possible to exercise control over the space where the molecules are released, the release time and the dose released (Figure 12).

The outbreak of desired activity (e.g. the release of an active principle or imaging localization) is made possible by the design of polymers that correspond to the target stimulus. The biophysical properties[179] most promising for biomedical applications include thermal sensitivity. This property is particularly useful for treating tumors because of the beneficial effects of a temperature increase on the destruction of cancer cells. It is therefore easy to combine the two concepts to enable the release of a therapeutic action. This can be achieved by the external application of a heat source because the increase in temperature causes the passage of certain polymer segments of the state of aggregates to the monomer, which releases the polymer network and allows for the distribution of the active

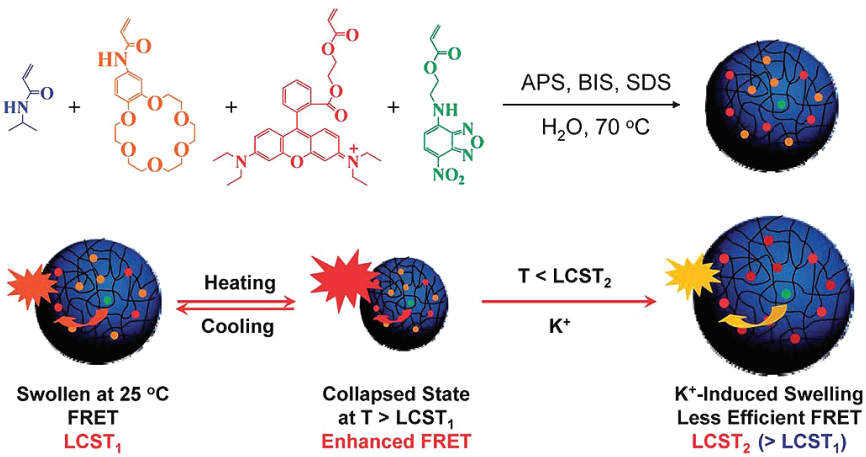

Figure 12. Example of the activation of an intelligent nanovector. The change in temperature causes a reduction in the size of the particle, which enhances the Förster resonance energy transfer (FRET) effect. (Taken from Ref. [178] with permission of Royal society of chemistry).

ingredient outside of the nanocarrier.[180] This behavior is possible for polymer units exhibiting a "lower critical solution temperature" (LCST). A significant number of studies have also included the activation of these polymer blends with the release of heat produced by the magnetic induction SPIO.[181–183] SPIO contributes to both the response to the stimulus and the component imaging. This property is often referred to as magneto-sensitivity. The LCST is the critical temperature below which the components of a mixture become miscible in any proportion.

Another important biophysical property is pH sensitivity. The observations that tumor microenvironments have a slightly acidic pH (pH = 6–6.5) and that acidification occurs during the endosomal and lysosomal digestion process (pH = 6–4.5) led to the design of pH-sensitive polymers. The effectiveness of this strategy for the targeted release of active ingredients has already been demonstrated *in vitro* and *in vivo*.[184–186] It is particularly advantageous in the case of gene therapy because the nanocarrier is able to disassemble at a specific pH and therefore avoid lysosomal degradation for better transfection of

target cells.[187] The release of the active principle is based on photocrosslinking of the polymer matrix, which induces tightening the polymer network and the subsequent expulsion of the encapsulated molecules.[188] Of course, other types of responses are being explored. These various strategies are an innovative and promising approach to circumvent the current blockages in the clinical translation of nanotechnology.

IV. How do Nanoparticles Pass Barriers?

An efficient drug delivery system needs to protect drugs from enzymatic, mechanical, or chemical degradation. It must also have enhanced diffusion through the epithelium, targeted tissue distribution, or increased penetration into the target cells.[189] Therefore, drug delivery nanosystems need to be designed to overcome many physical barriers.

A typical application that involves crossing biological barriers is the development of nanosized carriers for brain cancer treatment. The main limitations are crossing the blood brain barrier (BBB), the transport within the interstitium, the specific targeting of tumor cells and the delivery of the drug in high amounts.

To overcome these problems, controlling the physicochemical properties of the nanocarriers, such as composition, size, and zeta potential, is very important. The nanoparticles have to be smaller than 6–8 μm (the size of human red blood cells). To be sequestered inside the cellular nucleus, nanoparticles must have a diameter of less than 40 nm.[190,191] Unlike microparticles, nanoparticles delivered intravascularly can bypass the innate immune system by preventing uptake by the mononuclear phagocyte system (MPS).[192] Their size allows good penetration through the smallest pores of the capillaries in the human vasculature (200–1000 nm).[192–196] Spherical nanoparticles promote better surface coverage by hydrophilic polymers and targeting ligands, which is important.[197,198]

An ideal particulate carrier would have the capability to carry therapeutic agents to the target because of conjugated antibodies or other recognition moieties,[199,200] to image diseased tissue, and to

avoid biological barriers that can promote clearance from the systemic circulation.[201,202]

Healthy tissues contain blood vessels lined by a smooth layer of endothelial cells with pericytes that maintain the integrity of the vessels.[203] In tumor vasculature, there is a defective endothelial cell barrier with a loose attachment of pericytes that results in a leaky vasculature with fenestrations and irregular vessel diameters.[204] Many tumors lack lymphatic vessels, and tumors with lymphatic vessels have characteristically wider lumens and an increased number of intracellular spaces and sprout endothelial cells, resulting in an increase in interstitial fluid pressure.[203,205,206]

A tumor microvasculature has a characteristic pore cutoff size,[195,207] which is important for the design of a selective colloidal carrier. Transport across the tumor microvasculature has been shown to occur via interendothelial junctions, fenestrations and phagocytosis.[195,204] In normal vessels, interendothelial junctions have an effective size of 6–7 nm, which provides resistance to particulate drug delivery.[195,208] Hobbs *et al.*,[195] demonstrated that tumors grown subcutaneously have a tumor-dependent pore cutoff size of 200 nm –1.2 mm. Tumor microvessels have been shown to be hyperpermeable to long-circulating PEG liposomes and polystyrene latex particles of up to 600 nm in diameter.[207] The results obtained by Hobbs, Yuan, and Dreher demonstrated that the size of the nanoparticle should be within a range of 100–780 nm to effectively transport across the microvascular wall into the tumor interstitium.[195,207,209]

Drug delivery can be achieved by passive targeting (following the EPR effect, which promotes the accumulation of drug delivery carriers in the tumor interstitium)[210] or by active targeting because of specific receptors overexpressed on the surface of cancer cells;[211,212] molecules or ligands (e.g. antibodies, lectins, saccharides, hormones, and small molecular weight compounds) can recognize the cellular receptors to trigger internalization via receptor-mediated endocytosis.[211,213]

Drug delivery carriers can be externally guided to cancer cells or tissues with an applied magnetic field through which colloidal carriers bypass physical and cellular barriers. Strategies that incorporate iron

oxide nanoparticles and gold nanoparticles within the nanocarrier structure are beneficial for applications in magnetic resonance imaging (MRI), triggered drug release, and localized thermal therapy.[214–217] The concept of a magnetically targeted delivery system emerged 50 years ago,[218] and since then, extensive research has been performed to illustrate the utility of these ferrofluids in cancer treatment.[213] Once the particulate drug delivery has collected in the tumor vasculature, external activation by focused ultrasound, radiofrequency, laser light (photodynamic therapy), or an applied magnetic field can help trigger drug release and cell death (thermal ablation therapy).[219–222] Drug delivery carriers, such as liposomes (MAGfect)[215] and hollow microcapsule-loaded magnetite particles,[223] have been shown through MRI imaging to enable triggered release for anticancer applications.

To deliver high concentrations of drug to the brain, the BBB has to be crossed over by the nanocarriers. This barrier ensures tight regulation of transport between the bloodstream and brain tissue. Several methods have been explored to allow transport of a therapeutic across the BBB.[224]

One method to increase drug delivery is the use of BBB disruption to improve the permeability of the vessels by increasing the local osmotic pressure in brain vasculature using hyperosmotic agents.[225] However, this method does not provide the same results for all patients.[226] All BBB disruption methods cause decreased integrity of the complete BBB and not specifically the vasculature of the tumor, which could adversely affect healthy brain tissue. BBB disruption can also allow leakage of unwanted molecules from the circulation into the brain.[227] To avoid disrupting the entire BBB, various strategies have been employed to penetrate the BBB via nanoparticle drug carriers.

LDL receptors on the endothelial cells of the BBB can facilitate the uptake of nanoparticles coated with ligands for the LDL receptor. Xin *et al.*,[228] reported that Angiopep-coated PEG–poly(ε–caprolactone) nanoparticles can be accumulated in the tumor due to the passive EPR effect and active targeting through Angiopep. Apolipoprotein-coated particles using apoA-I-coated protamine/oligonucleotide nanoparticles *in vitro*[229] and apoE-coated serum albumin nanoparticles

in vivo[230] are taken up by binding to the LDL receptor. By grafting an agent that causes absorptive uptake on the nanoparticle surface, this study showed the uptake of drug-containing nanoparticles by the BBB endothelium via adsorption by the cells rather than by increasing gaps between endothelial cells in the entire brain. In another study, Kreuter *et al.*,[231] developed poly(butyl cyanoacrylate) nanoparticles as carriers for a peptide that normally could not cross the BBB. By coating the particles with a surfactant that caused apolipoprotein deposition onto the nanoparticles once they were in the plasma, they achieved absorptive uptake of their particles. The use of polysorbate 80[232] and other surfactant coatings, such as poloxamer 188[233,234] and Tween® 80,[235] has also been shown to cause high uptake by BBB endothelial cells in *in vitro* models and to lead to higher accumulation in the brain *in vivo*. Liposomes have also been cited to increase BBB penetration.[236] Other lipid-based nanoparticles have been reported to have BBB-penetrating properties as well as the ability to take advantage of the EPR effect in tumor models.[237]

To exploit the EPR effect or induce internalization by endothelial cells, particles being studied as potential drug carriers to brain tumors are generally less than 150 nm in diameter.

Another advantage of nanoparticle drug carriers in BBB penetration is their chemical versatility. Surfactants or stealth coatings, such as PEG, ligands and other biological or chemical moieties, can be conjugated to the surface to promote active uptake by cells on the luminal side, trafficking of the particle through the endothelial cell, and exocytosis into the brain tissue. For example, PLA nanoparticles were surface modified with PEG for stability and with cationic serum albumin for increased circulation time. The cationized albumin facilitated the interaction of the particles with brain endothelial cells to promote uptake with little or no observed toxicity.[238]

Qin *et al.*,[239] used Tat-conjugated cholesterol to formulate liposomes that showed the ability to transcytose through brain capillary endothelial cells and accumulate in the brain. In another study, poly(amidoamine) dendrimers were conjugated to a peptide derived from the rabies virus glycoprotein, RVG29, through a PEG linker and conjugated with DNA to form nanoparticles. The RVG29-modified

accumulated in the brain.[240] Other researchers have taken advantage of toxins that increase vascular permeability, such as the diphtheria toxin.[241] Specificity can also be built into the delivery system by using specific ligands that promote receptor-mediated endocytosis. Insulin was transported across the BBB from the circulation by receptor-mediated transcytosis,[242] and an antibody to the insulin receptor was taken up effectively into the brain *in vivo* in a primate model.[243] Ulbrich *et al.*,[244] conjugated drug-loaded human serum albumin nanoparticles to antibodies against insulin receptors and were able to achieve uptake into the brain in a mouse model. Polyester nanoparticles loaded with anticancer drugs, such as taxols, based on materials such as PEG-conjugated PLA or PLGA,[245,266] were able to achieve higher accumulation in brain endothelial cells when conjugated to transferrin. Like transferrin, the folate receptor for the transport of folic acid is also upregulated in rapidly dividing cells, including those in malignant brain cancer.[247]

V. Controlled Release from Mesoporous Silica Nanoparticles (MSN)

In order to have an ideal cancer therapy with nanoparticles delivery system, there is a need for nano delivery system that keeps anticancer drugs inside the nanoparticles until they reach tumor and the cargo (which is the anticancer drug in this case) is released only when a signal to release is provided. An important feature of nanoparticles which is used in cancer therapy is "zero release until nanoparticles reach tumor."[248] A variety of triggers such as external magnetic field, pH and redox state as well as external cues such as light have been used to accomplish on-command release. Besides carrying out delivery of anticancer drugs, they can transport compounds such as gadolinium complexes and fluorescent dye. Therefore the probe changes to the theranostic agent which can have the imaging and therapy simultaneously.[248,249]

Mesoporous silica nanoparticles are usually synthesized by a sol–gel method to produce homogeneous size nanoparticles.[249]

Mesoporous structures which have a structure with many small pores are the results of addition of surfactant during the synthesis of these nanoparticles. Kuroda and his colleagues are the pioneer and developer of the idea of surfactant template to produce mesoporous materials.[250]

Mesoporous silica nanoparticles (MSNs) have numerous advantages for developing controlled release systems. Firstly, extensive surface area enables various modifications to be carried out. Secondly, they are relatively more stable in comparison with other types of NPs. A variety of chemical modifications have been made on their surface as well as on pore interiors. Thirdly, the pores in the NPs provide storage space for anticancer drugs, therefore these NPs have a great ability in loading.

A. Mesoporous silica nanoparticle (MSN)-based controlled release systems

There are two main approaches to obtain MSN with control release capabilities. The first approach which was developed by Stoddart and Zink who involve attaching organic molecules (such as rotaxanes and pseudorotaxanes) at the pore opening in order to preventing release of the cargo (here it could be anti-cancer drugs) stored in the pore.[251] This method is a so-called "capping" or "gating." "Nano-valves" can be attached to the pore openings to provide open and close function for the cargo stored in the pores. The anticancer drugs stored in the pores will remain inside nanoparticles by closing the nano-valve. Various other materials have been developed for capping (gating). Polymers have also been used to cover pore openings.

The second approach to prepare MSN with controlled release feature is to attach drugs to the surface of MSNs via stimuli-responsive linkages.[249] Two kinds of stimuli can be employed. Firstly, MSNs that respond to external stimuli such as an external magnetic fields[251–258] or light (such as near infra-red (NIR) or ultra violet (UV)) which leads to increase the temperature of environments[259] have been developed. Use of ultrasound to trigger drug

release has also been explored.[260,261] Studies showed that using magnetic field as external stimuli for controlled release has an advantage, as it has much better tissue penetration compare to the light.[249,262] MSNs can be heated up to 42° while maintaining the surrounding temperature at 19°.[252,253] The second stimuli are intracellular or intratumoral conditions which is called autonomous release of anticancer drugs. These triggers could be enzymes, biomolecule[263–266] or low pH (due to hypoxic conditions in the tumor where the pH is low). This feature provides an advantage that the drug release is more limited to the tumor area).[267–271] Redox and biomolecule-activated systems (reducing conditions such as glutathione)[272–277] have also been used.

For more detailed information on chemical features of these and other nanoparticles, please refer to reviews by Mekaru and

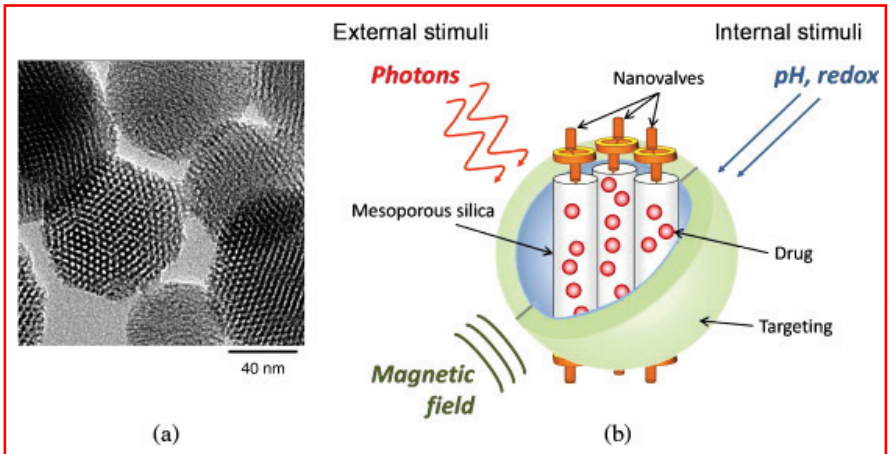

Figure 13. Mesoporous silica nanoparticles synthesized by the sol–gel method. (a): TEM of MSNs. They are homogeneous with the diameter of approximately 130 nm and contain 1400 pores that can be used to store anticancer drugs. (b): A schematic overview of mechanized nanoparticles based on MSN. Nano-valves will be attached to the opening the pores. Cargo molecules such as anticancer drugs and dyes can be stored in the pore. Surface modifications can be done to target tumor. Nano-machines that respond to internal stimuli such as pH and redox as well as external stimuli such as light and magnetic field will be used to operate nano-valves.[249] (Taken from Ref. [250] with permission of Elsevier).

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Tamanoi,[249] Song and Yang,[278] Victor Lin *et al.*,[279] Chen *et al.*,[280] or Duguet *et al.*[281]

VI. Controlled Release from SPIONs

As explained before, since using external magnetic field as a trigger, controlled release had a better penetration depth in comparison with using external light. In order to reach this advantage, it is necessary to use a special type of nanoparticles that contains iron oxide magnetic core.[262] These nanoparticles have usually a core size of 2–10 nm which contains iron oxide (Fe_3O_4). A mesoporous silica coating was added to make a particle of about 100 nm.[249] This magnetic core gives numerous properties to the nanoparticle. Firstly, due to the superparamagnetic property of iron oxide core, the nanoparticles have the capabilities to be heated up by the exposure to oscillating magnetic field. Secondly, the magnetic property can be used to recover nanoparticles or to collect them to a desired site. Thirdly, iron oxide has a property to enhance MRI imaging contrasts using T_2 weighted imaging protocols.[282,283]

Due to the superparamagnetic property of iron oxide NPs, exposure of iron oxide core of NPs to alternative magnetic field results in heat generation. This temperature increase can be used to allow opening of a nano-valve.[249] To accomplish this, a special type of nano-valve consisting of a stalk and cucurbituril was synthesized. The valve becomes open after the heat generated by the external magnetic field.[255,284,285]

For more detailed information on chemical, and physical features of these nanoparticles, please refer to reviews written by Mekaru and Tamanoi[249] or Kim and Nguyen.[286]

VII. Controlled Release from Membranes

In medicine, encapsulated drug is release in the body after it is administrated orally or intravenously.[287] Controlled release reduces the loss of an expensive active agent due to leaching at an unnecessarily high rate and eliminates its excessive dispersion to the environment. There

are many factors which may affects the rates of drug released by the membrane. Tojo *et al.*,[287] investigated the effects of the void fraction, membrane thickness, concentration dependence of diffusivity, and step change in the temperature of the environment on the rate of release. They showed that particles with an excess active agent in the core zone have the profoundly desirable controlled released characteristics. During the past decades, controlled released technology as a new multidisciplinary science has received increasing attention in the pharmaceutical and medical sciences.[288] The unique permeation characteristics of polymeric membranes have begun to be applied for controlled delivery of biologically active agents.[289] It has been predicted that the major controlled release technology will be the polymeric membrane type.[288]

Diffusion controlled membrane tools can be divided into two main categories: the first one is reservoir systems (the active agent is totally encapsulated within a rate controlling membrane) and the second one is monolithic systems (which the active agent is dispersed or dissolved in a rate controlling matrix).[290]

VIII. Controlled Release from Hydrogels

Hydrogels are polymeric networks with three-dimensional configuration. They can absorb bulky quantities of water or biological fluids while remaining insoluble in aqueous solutions due to chemical or physical crosslinking of individual natural or synthetic polymer chains.[291,292] Their attraction to absorb water is because of the presence of hydrophilic groups such as –OH, –CONH–, $–CONH_2$, and $–SO_3H$ in polymers forming hydrogel structures.[293]

The unique physicochemical characteristics are determined by the water content of a hydrogel. Some of their physical properties such as their high water content, their soft and robbery consistency, and low interfacial tension with water or biological fluids resemble the living tissues, than any other class of synthetic biomaterials.[294]

However, hydrogels show a swelling behavior instead of being dissolved in the aqueous surrounding environment as a consequence of the critical cross-links present in the hydrogel structure.[295] The water

content in the hydrogel depends on the distance between polymer chains and the flexibility of those chains together.[296]

Hydrogels can be classified based on a variety of characteristics, including: the nature of side groups (neutral or ionic), mechanical and structural features (affine or phantom), method of preparation (homo- or co-polymer), physical structure (amorphous, semicrystalline, hydrogen bonded, supermolecular, and hydrocollodial), and responsiveness to physiologic environment stimuli (pH, ionic strength, temperature, electromagnetic radiation, etc.).[292,297]

The polymers commonly used in preparation of hydrogels with pharmaceutical and biological applications have natural or synthetic origins.[297] Therefore, there is a classification based on the type of polymeric materials[295]:

1. Chitosan-based hydrogel nanoparticles
2. Alginate-based hydrogel nanoparticles
3. Poly (vinyl alcohol)-based hydrogel nanoparticles
4. Poly (ethylene oxide) and poly (ethyleneimine)-based hydrogel nanoparticles
5. Poly (vinyl pyrrolidone)-based hydrogel nanoparticles
6. Poly-N-isopropylacrylamide-based hydrogel nanoparticles
7. Hydrogel nanoparticles of other origins

In general, there are three important parameters in describing the nanostructure of cross-linked hydrogel networks: (1) polymer volume fraction in the swollen state, (2) number average molecular weight between crosslinks, and (3) network mesh size, zeta potential ξ.[298]

Drug release mechanisms from hydrogels can be categorized as:

A. Diffusion-controlled

The most common feasible mechanism for describing drug release from hydrogels is diffusion-controlled. Molecules of different sizes and characteristics can freely diffuse into/out of hydrogel matrix during the loading and storage periods.[295]

The drug diffusion out of a hydrogel matrix depends on the mesh sizes within the matrix of the gel, which is affected by several parameters. Typical mesh sizes reported for biomedical hydrogels range from 5 to 100 nm (in their swollen state), which are much larger than most small-molecule drugs.[295,299] Fick's law of diffusion with either constant or variable diffusion coefficients is commonly used in modeling diffusion-controlled release.[300]

B. Swelling-controlled

This mechanism is considered as a releasing behavior, when diffusion of a drug is significantly faster than hydrogel distention. The modeling of this mechanism usually involves moving boundary conditions where molecules are released at the interface of rubbery and glassy phases of swollen hydrogels.[299]

C. Chemically-controlled

Chemically-controlled release is used to describe molecule release determined by reactions occurring within a delivery matrix. The most common reactions that occur within hydrogel delivery systems are cleavage of polymer chains via hydrolytic or enzymatic degradation or reversible or irreversible reactions occurring between the polymer network and releasable drug.[296]

For a successful drug delivery, controlled-release is needed to release the drug of interest at a specific predetermined temporal and/or spatial manner within the body. Therefore, there are several studies about controlled-release systems. The hydrogel-based delivery systems are of two major categories: i) time-controlled systems and ii) stimuli-induced release system.[295,296]

Most of controlled-release systems use stimuli-sensitive hydrogels however their response time is considerably slow. For fastening their response, developing thinner and smaller hydrogels could be effective. But this approach causes fragility and loss of mechanical strength in the polymer network.[301]

The stimuli-sensitive hydrogel systems can be further sub-classified into[302]:

i) Physically-induced release systems: Temperature, electricity, light, pressure, sound, and magnetic field.
ii) Chemically-induced release systems: pH, solvent composition, ions, and specific molecular recognition events.
iii) Other stimuli-induced release systems.

Investigations on other controlled release systems from hydrogels and developing ideal system to achieve the most precise drug delivery are continued.

IX. Controlled Release from Lipid-Protein NPs

Traditional administration routes for drugs are the oral and parenteral administration. In both routes, the drug arrives in the blood stream finally. After that, it will be distributed in the body depending on its physicochemical properties. Therefore, it can be degraded. For the solution of these problems and having targeting, a new approach was suggested: the entrapment of those drugs into a particulate carrier system. One of the carrier systems is lipid nano-emulsions, They are fine oil/water (o/w) dispersions, having droplets covering the size range between 50 and 200 nm for carrying lipophilic drugs.[303] Lipid-like nanoparticles can be protein and gene delivery vehicles and also for RNAi.[304,305] These lipid carriers may be available in different phases such as solid, semi-solid, or liquid state in the form of solid lipid nanoparticles, nanostructured lipid carriers, lipid drug conjugate nanoparticles, liposomes, or nanoemulsions.[305] They can reduce side effects but are thermodynamically unstable.[306] Among the lipid carriers, liposomes are well established and extensively investigated. They are able to control release successfully.[306] Some of the advantages of liposomes are compatibility of the constituent components with the body system thereby presenting low inherent toxicity, facile preparation and easy variation of composition to obtain more efficient preparations.[306]

Solid lipid nanoparticles (SLN) were described by Müller *et al.*,[307] for the first time. They have been introduced as an efficient and non-toxic alternative lipophilic colloidal drug carrier. Two established production techniques are: the high-pressure homogenesation described by Müller and Lucks[308] and the microemulsion-based technique by Gasco.[309]

They allow drug protection and administration via parenteral and non-parenteral routes thus emphasising the versatility of this nanoparticulate carrier because of their colloidal dimensions and the controlled release behaviour.[306]

X. Toxicity of Nanoparticles

Despite our exposure to nanomaterials is growing, there is little understanding of the unique toxicological properties of NPs and their long-term impact on human health. Nanomaterials can be released into the environment via spillages, wear, washing and disposal at a rate proportional to their level of use. The worst thing is that we don't know where they go or what happens to them after they are released. Because of their very small size, they might enter the human body by inhalation, ingestion, skin penetration or injections, and NPs can interact with intracellular structures and macromolecules for long periods of time.[310] Whereas transition from bulk materials to nanomaterials cause changing the properties, the toxicity of a bulk material may not be a good indication of the toxicity of the nanomaterial. Moreover, toxicity is already hard to measure because it depends upon dose and the animals, plants or cells tested. Although there are a lot of studies about the toxicity of variety nanomaterial compounds. Some of them compared two types of toxicity measurements, *in vitro* and *in vivo*. This comparison demonstrated that for identifying characteristics of nanomaterials that can be used as indicators of toxicity and in order to establish a ranking of NP toxicity for mechanistic studies, the *in vitro* systems are principally practical. But for studying aspects that cannot be obtained with *in vitro* systems, such as toxico-kinetics in the body, i.e. absorption, distribution, metabolism, and elimination, the *in vivo* tests would be mainly. However,

the disadvantages of *in vivo* measurements are time-consuming, expensive, and involve ethical issues.[311]

The another priority of *in vitro* methods in comparison with *in vivo* methods is that they can produce reproducible results rapidly and inexpensively without the use of animals.[312]

Among all *in vitro* toxicity assays, the MTT assay method has been prominent for probing the toxicity of SPION.[313] It allows rapid evaluation of cell viability, cell survival, cell growth and gives good reproducibility.

The physicochemical properties such as size, surface chemistry, shape, protein absorption gradient and surface smoothness or roughness play a critical role in determining the toxicity of nanomaterials. Consequently, a fundamental understanding of the biological interactions of NPs with cells, proteins, and tissues, is vital to the future design of safe nanotechnologies. The main concern is a high degree of biocompatibility of NP-products besides minimum negative effects on blood components, genetic material, and cell viability.[310]

XI. Protein Corona and Its Effects on Targeting Capability of Nanoparticles and Their Drug Release Profile

Studies showed that, as the NPs enter in the biological medium, the surface of NPs is covered by various biomolecules (proteins) through a process called "protein corona effect."[314,315] Thus, the primary interaction of organs or cells with NPs is strongly influenced by the "hard corona" (i.e. a long-lived protein layer that strongly adsorbed to the surface of the NP and remains stable for several hours).[316,317] This new biological identity of the NPs can entirely change the biological fate of the NPs. Therefore, it is important to have a deep understanding on the interactions at the nano-bio interfaces to design safe, reliable, and high-yield NPs, for desired biomedical purpose. In this case, extensive studies were dedicated to probe every individual crucial factor, which can be considered at the nano-bio interfaces.[318,319] For instance, it has been discovered that the protein corona can cover the targeting molecules on the surface of NPs and causes loss of specificity

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in targeting.[320] Mahmoudi *et al.*,[321] showed that the corona formation slows down the fibrillation process of the amyloid proteins (e.g. amyloid beta), regardless to the physicochemical properties of the NPs. Another study showed that after getting very promising result in *in vitro* the *in vivo* results were disappointing due to the coverage of targeting site by protein corona and because absorption of opsonin-based proteins at the surface of nanoprobes prohibit the active targeting and the probe was eliminated from the blood immediately after injection by the RES system.[322]

Mahmoudi *et al.*,[318] showed that the drug release profile of nanoprobes depends on their interaction with the protein corona (i.e. the amount and types of the associated proteins in the composition of hard corona) in addition to the size/type of the nano-probe. The formation of a protein buffer layer onto the surface of small nanoparticles *in vivo* acts as a shield and causes a delay in the drug release process and this effect plays a crucial role in the *in vivo* applications. They concluded that, the release profile data that are currently available for various drug-carriers can be modified by incorporating the protein corona effect to have realistic *in vivo* applications.

XII. Future Perspective on Smart Drug Delivery Systems

A smart system is one that can alter its properties in response to environmental changes, such as pH, temperature, enzymes, and ionic environment. Ideally, a drug delivery system needs to perform multiple functions, including improving the solubility and stability of the drug/payload, reducing the dosage and its frequency, and reducing or eliminating the drug's adverse effects. The carrier should be nontoxic to the biological system, should not trigger adverse immune responses, and, finally, *should be able to deliver the required amount of drug to the desired location over a long period of time*. This last point is the most challenging for a smart drug delivery system (SDDS), which is also called a stimuli-sensitive delivery system.[323]

Two aspects are important. First, the surface modification with a suitable targeting vector on the nanosystem surface delivers the drug at the desired location, and second, the presence of a trigger that can

control the amount of drug released at a given instant ensures delivery of required amount of the drug. The signal can be due to an external stimulus (extrinsic triggers such as ultrasound, magnetic or electric field) or to the environment of the system itself (internal triggers such as temperature, proteins, carbohydrates, and pH).

An SDDS has several advantages compared to a conventional drug delivery system (DDS). A DDS release the same amount of the drug independent of the environmental conditions, while an SDDS is based on the release-on-demand strategy, which allows a drug carrier to liberate a therapeutic drug only when it is required in response to a specific stimulation.

Three categories of polymeric systems can be employed exhibit stimuli responsiveness, including linear polymeric chains, cross-linked gels and surface grafted systems.

The solubility of linear polymeric chains can change with a stimulus due to alterations in their hydrophobicity and hydrophilicity. In an aqueous medium, if the stimulus induces an increase in the hydrophilic interactions, the polymer swells, while if the stimulus increases the hydrophobicity, the polymer chains collapse and precipitate out of the solution.

The cross-linked gels exhibit rapid responses to small changes in the stimulus. However, caution should be exercised when tightly regulating the extent of cross-linking because over-crosslinking will lead to a loss in the swelling and deswelling property.

For grafted polymer chain systems, the swelling or deswelling can contribute to a variation in the hydrophilicity or hydrophobicity of the substrate. Such a phenomenon depends on the changes produced at the interface between the polymer chains and the environment and is now referred to as "***interfacial engineering***."

A typical example of an SDDS is a self-regulated insulin delivery systems that can respond to changes in the environmental glucose level.[324,325]

Among the numerous SDDSs available, one of the most widely used is polymeric micelles, which can dissolve water-insoluble drugs, such as doxorubicin or paclitaxel, at high concentrations.[326–328] In the body, drug release from micelles depends on the simple diffusion and

degradation of the micelles. The kinetics of drug release can be modulated by varying the degradation rate of hydrophobic polymers; however, the degradation rate is usually very slow, and the drug is released by diffusion from micelles. This release by passive diffusion may be undesirable because the polymeric micelles reaching the target site need to release their contents quickly. To avoid this slow release, smart polymeric micelles have been developed to liberate the loaded drug at the targeted site more quickly. For example, Lee *et al.*,[329] described poly (ethylene glycol)-b-poly-histidine (PEG-b-PHis) micelles that are stable only over the pKb of the poly-histidine block (pH 6.5–7.0). The pKb can be adjusted by varying the molecular weight of poly-histidine. Because solid tumors have a slightly acidic environment, a small reduction in pH at the tumor site triggers a dissociation of the polymeric micelle to release its contents. The same authors[330] reported that PEG-b- poly-histidine micelles containing doxorubicin killed multi-drug resistant MCF-7 cells at pH 6.8. Similarly, Hruby *et al.*,[331] showed that an SDDS can achieve highly localized drug accumulation at target sites.

An SDDS with enhanced targeting properties is highly promising for increasing the efficiency and efficacy of therapy while decreasing side effects.

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