several workers [58–61]. Only a proportion of the colon will be usable for drug delivery, unless prepared by irrigation, and the useful sites will generally include the cecum and ascending and transverse colon. The length of time available for drug release in these regions of the gut, and the influence of formulation variables (size, shape, and density) on transit in "normal" and pathophysiological conditions, is therefore of great interest to pharmaceutical scientists. There are conflicting values in the literature regarding transit from cecum to splenic flexure; for example, in a study quantifying the change in geometric center in normal and slow transit, the value was approximately 15 h in normal subjects and 52 h in patients with slow colonic transit [62].

Gamma scintigraphy was used to compare the colonic transit rate of different sizes of nondisintegrating radiolabeled model dosage forms in healthy subjects. In the first study, colonic transit of radiolabeled capsules, volume 0.3–1.8 cm^3 and density 0.7–1.5 g/cm^3, were monitored in 18 healthy subjects. The capsules were administered after an overnight fast and entered the colon, on an average, 5 h after dosing. Transit rates through the proximal colon were independent of capsule density. Effects due to capsule volume were small compared to intersubject variations in transit rates. Within 10 h of entering the colon, 80% of the units had reached the splenic flexure [63].

By administering both sizes of formulation simultaneously, a better discrimination of relative transit of the two phases can be made. In a cohort of 22 healthy young volunteers, an enteric-coated capsule was administered, which contained tablets ([^{99m}Tc]-labeled; 5 or 8.4 mm in diameter) together with pellets ([^{111}In]-labeled 0.2 mm ion-exchange resin particles). The unit delivered the radiopharmaceuticals simultaneously to the ileocecal junction [64]. Under controlled conditions, no difference was observed between the rate of transit through the ascending colon of 0.2 mm particles versus 5 mm tablets or 0.2 mm particles versus 8.4 mm tablets. The mean period of residence of 50% of the administered 0.2 mm particles in the ascending colon was 11.0 ± 4.0 h.

Adkin and colleagues compared transit of 3, 6, and 12 mm nondisintegrating [^{111}In]-labeled tablets in eight healthy male volunteers. The transit of tablets through the ileocecal junction was unaffected by tablet size. All tablets entered into the colon as a bolus. The 3 and 6 mm tablets were retained in the ascending colon for the longest period of time [65].

21.5.2 Time of Dosing

The time of dosing is an important parameter with regard to the whole-gut transit of nondisintegrating formulations. Sathyan and colleagues [66] have noted that an analysis of 1163 administrations of OROS devices showed a bimodal distribution clustered at 12 and 36 h following nighttime dosing and 24 and 48 h after morning dosing. After nighttime dosing, a monolithic device will be propulsed forward during the strong contractile activity on waking and rising, but may not move far enough to be excreted. Our own data show that, subsequent to the mass movement in the first hour of rising, propulsive movements are weak until sometime after the lunchtime meal has been ingested [67].

21.5.3
Modulating Colonic Water

In our early attempts at producing models of diarrheal predominant disease, it was found that the administration of the laxative lactulose provided a useful and reversible simulation of altered colonic hydrodynamics such as might be seen in colitis. Lactulose is used therapeutically to manage a number of conditions including hepatic encephalopathy, constipation, and salmonellosis. This semisynthetic disaccharide is neither metabolized nor absorbed in the normal small intestine, but may undergo bacterial fermentation in the colon to short-chain fatty acids and gases. Major consequences include a fall in pH and a change in the composition and metabolic activity of the colonic flora [68]. The changes provoked by lactulose are sensitive to fiber supplementation [69] and can be reversed by codeine [70].

In the experiments with enteric-coated tablet plus pellet preparations [64], a second leg was conducted in which a preparation containing 5 mm tablets and 0.2 mm resin was administered after laxative treatment. Following lactulose dosing, there was a significant acceleration in colonic transit and the ascending-colon residence time of the 0.2 mm resin was significantly shorter than that for the 5 mm tablets, though the magnitude of the effect was small.

In later experiments, stool water content was modulated and the influence of luminal water content on the absorption from the distal gut of either quinine (a transcellular probe) or [^{51}Cr]-EDTA (a paracellular probe) was observed. Absorption of these probe markers from a timed-release delivery system was determined following treatment with lactulose 20 ml t.d.s. (increasing water content) or codeine 30 g q.d.s. (decreasing water content) and compared with control untreated values. Lactulose accelerated ascending-colon transit, increased stool water, caused greater dispersion of released material, and enhanced the absorption of the quinine compared to control. Conversely, codeine slowed down ascending colon, reduced stool water content, and also tended to diminish absorption. More distal release resulted in less absorption in the control arm, whereas lactulose enhanced drug absorption from the distal gut [71]. An interesting finding was that a proportion of the asymptomatic normal volunteers showed higher than expected urinary recoveries of [^{51}Cr]-EDTA (5–10% of dose) suggesting an increased paracellular permeability.

Other workers have shown that the increased fluid load produced by an osmotic laxative results in redistribution of colonic contents. Since the distal colon is considered to be mainly a conduit without extensive storage function, Hammer and colleagues considered whether the capacity of the colon to retain fluid might be relevant in compensating for increased fluid loads and preventing diarrhea. Changes in distribution following cecal infusion of an iso-osmotic solution labeled with [^{99m}Tc]-DTPA containing polyethylene glycol (PEG; 500 ml) were compared with changes following infusion of an equal amount of readily absorbable electrolyte solution. After the osmotic load, fecal output was increased significantly ($p < 0.05$), but whole-colonic transit after PEG infusion was not different from transit after the

electrolyte solution ($p > 0.05$), indicating that the distal colon is able to manage nonabsorbable fluid volumes to a large extent [72].

21.6 Pathophysiological Effects on Transit

Active left-sided colitis is often resistant to topical therapy, and resolution may only be achieved by administration of systemic therapy. Twenty-two volunteers and ten patients were recruited for a clinical trial in which they received morning doses of a Eudragit-coated capsules containing [^{111}In]-labeled resin pellets [73]. At day 4 into the regime at steady state, the relative distribution of the marker was measured in the ascending, transverse, descending, and rectosigmoid colon. The results showed that colonic distribution among healthy subjects was asymmetric, with two-thirds of the administered dose in the proximal colon and one-third in the distal colon. In the patients, this difference was even more pronounced, with only one-tenth of the administered dose in the distal segment.

Rapid transit through this region suggests that the area is empty of colonic contents most of the time, and so the opportunity for topical treatment is consequently limited. If the exposure to a drug such as mesalazine is calculated on the basis of these data, the results show that treatment is probably inadequate. For example, the dose per day is approximately 3 g (800–1200 mg, t.d.s.), and therefore in active disease, the effective dose would be about 300 mg on the basis of this regimen. Doses of between 500 and 1000 mg are often given as an enema, but these doses are more effectively delivered and not sequestered within a viscous, partially dehydrated stool, as would be the case following oral administration. Modern "gold standard" treatment suggests that a combined oral and rectal dosing strategy is the most efficacious method of using this drug.

Although discrete effects of diseases are often noted by studying a single parameter such as gastric emptying, the effects of pathophysiological conditions, once established, are usually evident throughout the gut. Mollen and colleagues attempted to describe the motor activity of the upper gastrointestinal tract in patients with slow-transit constipation using perfusion manometry. Orocecal transit time was found to be similar between patients and controls, but esophageal motility was abnormal in 5 out of 18 patients and gastric emptying was abnormal in 8 out of 15 patients. These data support the case that disorders of upper gut motility occur frequently in patients with slow-transit constipation [74]. Gattuso studied 10 young patients with idiopathic megarectum using radiographic and scintigraphic methods. All patients had a dilated large bowel, with no radiographic evidence of upper gut dilation. Gastric emptying was normal in four patients and abnormally slow in six, which suggested that this bowel condition might be reflected in a disturbance of upper gut function. Both radioisotope scans and radio-opaque marker studies showed abnormal colonic transit, and regions of delay corresponded with the region of dilated bowel [75].

Studies in dogs have shown that postoperative ileus following surgery resolves an initial phase of weak irregular, nonpropagating contractions of the gastrointestinal tract, followed by transmission of the contractions from the upper gut to the lower gastrointestinal tract. Tsukamoto [76] found that recovery from postoperative ileus was aided by a change in the pattern of gastrointestinal motility in which contractions were transmitted from the stomach to the lower gastrointestinal tract, like an interdigestive migrating contraction. Bouchoucha characterized colonic transit time in 30 healthy subjects and in 43 patients with inflammatory bowel disease using X-ray opaque markers. The response to food was different in the two populations: in controls, the cecum and ascending colon emptied and filled the distal bowel, whereas in patients, only the splenic flexure and left transverse colon emptied. Movement through both the right and the left colon in patients was observed to be much slower than that in controls, both before and after a meal [77].

Patients with anxiety and depression often have bowel symptoms. Gorad and colleagues compared 21 psychiatric outpatients with generalized anxiety disorders and depression with an equal number of healthy controls. Whole-gut transit time (WGTT) was found to be shorter in patients with anxiety (mean 14 h; range 6–29 h) than in either those with depression (mean 49 h; range 35–71 h; $p < 0.001$) or controls (mean 42 h; range 10–68 h; $p < 0.001$). In patients with anxiety, orocecal transit time (measured using the lactulose hydrogen breath test) was shorter than in patients with depression and also shorter than in controls. The authors concluded that anxiety is associated with increased bowel frequency, while depressed patients tend to be constipated; taken together, these data strongly suggested that mood has an effect on intestinal motor function [78]. Bennett and colleagues [79] concluded that male hypochondriacs had normal intestinal transit, whereas elderly females with depressive illness were more likely to have both colonic and gastric stasis.

Among several disease conditions that affect gastric emptying, diabetes is probably the most extensively studied. Folwaczny and colleagues used scintigraphy to examine esophageal transit and gastric emptying and a metal-detector test to determine large bowel motility in patients with type I and type II diabetes. These authors concluded that both gastric emptying and large bowel transit were affected by both conditions [80].

The alteration of transit by disease, or a change produced by hydrodynamic factors such as diarrhea, will be highly significant for sophisticated zero-order release formulations such as osmotic pumps. For the pumps, inadequate retention may occur in some patients, perhaps leading to less optimal clinical outcome. Even in normal subjects, the range of intestinal transit time can be extreme; for example, the median gastrointestinal transit time for both oxprenolol and metoprolol OROS drug delivery systems has been reported as 27.4 h, with individual times ranging from 5.1 to 58.3 h [81].

Hammer [82] conducted an experiment in which volunteers received either autologous blood or egg white by duodenal intubation to simulate the condition of upper gastrointestinal bleeding. [^{99m}Tc]-DTPA was added to each infusion and arrival at, and clearance from, the colon was recorded. At 4 h after the start of blood infusion, a median of 30% of counts was observed in the transverse colon compared to 0% after

egg white administration; small intestinal transit was unaffected. Although it had been established that bleeding alters gastric motility, this demonstration for the first time that haem-containing proteins have a significant effect on proximal bowel motility.

21.7
Pathophysiological Effects on Permeability

Inflammation leads to changes in permeability of large and polar molecules, which forms the basis of diagnostic tests such as urinary recovery of [^{51}Cr]-EDTA after oral administration. Evidence for increased permeability to very large molecules and small particles in humans is limited, although in an experimental model of colitis in the rat, Lamprecht [83] demonstrated significant uptake of 100 nm-sized particles compared to controls.

21.8
pH

The pH changes of the gut are obvious triggers for the delivery of drug from enteric-coated preparations, in particular, tablets used for the delivery of topical agents in the treatment of bowel disease. Sasaki and coworkers measured pH profiles in the gut in patients with Crohn's disease by using a pH-sensitive radiotelemetry capsule as it traveled from the stomach to the cecum. Gastrointestinal pH profiles measured in four patients with left-sided Crohn's disease were similar to those in four gender- and age-matched control subjects. In contrast, colonic luminal pH profiles in both right and left colon in active or quiescent Crohn's disease showed more coarse fluctuations, with significantly lower values than were seen in controls [84].

The bulk luminal pH is heavily affected by the fermentation of carbohydrates to short-chain fatty acids; however, near the colonic mucosa, the pH rises and changes in the bulk pH have little effect on the epithelial microclimate. Bicarbonate/chloride exchange is partly responsible for raising the pH against the challenge posed by the high colonic p_{CO_2} and the acid production by fermentation. The mucus has been shown to contain a distinct carbonic anhydrase, produced by epithelial tissues that help to carefully regulate the thick unstirred layer of the colonic epithelium.

Many patients with Crohn's disease undergo an ileocecal resection, which might be expected to influence small intestinal pH and transit time. A "radiopill" technique (similar to that of Sasaki *et al.*) was used by Fallingborg and colleagues [85] to examine intraluminal pH and transit time in ileocecally resected Crohn's disease patients. These data were compared with those obtained from 13 healthy volunteers. The mean SITT was significantly shorter in patients than in controls (5.2 and 8.0 h, respectively). However, although the pH levels of the small intestine were identical in patients and controls, cecal pH was 0.9 pH units higher in resected Crohn's disease patients, and the period when the pH was elevated above 5.5 was significantly shorter in patients than in controls [86].

21.9 Conclusions

In summary, it is emphasized that disease conditions may result in changed physiological parameters that could strongly influence the effectiveness of orally administered medications. Changes in patterns of motility, pH, and amount of water available for dispersion and dissolution may be significant for patients compared to the "normal" population. On this basis, it seems appropriate to completely investigate the impact of a target disease – whether it is diabetes, inflammatory bowel disease, or irritable bowel syndrome – on the deposition of drug from the candidate delivery system. Neglect of these issues might lead to suboptimal therapy and a waste of healthcare resources.

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22
Nanotechnology for Improved Drug Bioavailability

Marjo Yliperttula and Arto Urtti

Abbreviations

AUC	Area under the curve
BCS	Biopharmaceutical Classification System
CMC	Critical micelle concentration
DNA	Deoxyribonucleic acid
FDA	Food and Drug Administration
i.v.	Intravenous
PEG	Polyethylene glycol
SEAP	Secreted alkaline phosphatase
siRNA	Small interfering ribonucleic acid

Symbols

3D	Three-dimensional
CD44	Membrane receptor for the hyaluronan uptake into the cells

22.1 Introduction

Nanotechnology is one of the major fields of current pharmaceutical research. This field is strongly related to drug delivery and targeting, because nanosized drug delivery systems are able to modify the pharmacokinetics significantly. Based on the most common definition, nanotechnology is dealing with structures of below 1 μm. At very small sizes, material has peculiar physical chemical properties that are distinct from the macroscopic properties of the same matter. In pharmaceutical nanotechnology, these physical distinctions are not the main point. Rather, pharmaceutical nanotechnology aims to generate structural units below 1 μm and, most importantly, these particulates should have controlled functionalities that improve drug delivery.

Bioavailability is a parameter that defines the fraction of the dose that enters the "site of action." In general, bioavailability refers to the *systemic bioavailability*. After per oral drug administration, the systemic bioavailability is always 0–100%, and the extent of bioavailability is determined by comparing the AUC values of the drug concentration in plasma versus time curves after oral and intravenous (i.v.) (100% bioavailability) drug administration [1]. There are some cases in which systemic bioavailability is improved with nanotechnology (e.g., nanocrystals). However, the main emphasis in the design of nanosystems is to improve *local bioavailability*. For example, nanotechnology is used to increase drug or gene delivery into the diseased target tissue, for example, vessel wall, tumor, or brain [2]. Another therapeutic goal is to improve the therapeutic index. Doxorubicin liposomes are a good example [3]. The drug as such is cardiotoxic as a solution, but liposomal doxorubicin avoids the toxicity by maintaining the free drug concentration in the blood stream at low level. Because the liposomes are preferentially distributed to the tumor tissue, the overall therapeutic index and efficacy of the treatment are improved.

Nanotechnology is a timely topic in pharmaceutical science and there are good reasons for that. *First*, in drug discovery, the solubility of new chemical entities shows historically a declining trend. In fact, poor water solubility has become one of the major problems that limit the systemic drug bioavailability. Nanosizing has been used to improve the rate of dissolution, and sometimes this method results in improved drug absorption after oral dosing [4]. *Second*, some target sites of drug treatment are difficult to reach. For example, narrow therapeutic index is a problem in anticancer drug treatment, as these drugs have serious adverse effects. If the doses could be reduced in association with improved drug delivery to the tumors, the extent of side effects would also be reduced. Other difficult targets include the brain and the posterior segment of the eye. *Third*, emerging biotechnological pharmacologically active compounds require nanotechnology-based drug delivery. This is because biotechnological drugs, such as peptides, proteins, oligonucleotides, and DNA, have large molecular weight, and they are not able to distribute in the body as small molecules do. The task is further complicated by the susceptibility of these drugs to enzymatic degradation by peptidases and nucleases. In many cases, the target sites are intracellular (e.g., siRNA, transcription factors, and DNA), and the delivery system should protect the drug from these enzymes and shuttle it to intracellular target sites [5]. In this case, the goal is to improve bioavailability in the cellular target organelles of the diseased tissue. In fact, the progress of these therapeutic approaches depends on the possibility to efficiently deliver these compounds to the target sites. In the case of gene medicines, it is straightforward to find the drug (i.e., DNA or siRNA sequence) after biological basic research on the mechanisms of the disease. However, drug delivery becomes the limiting factor.

Nanotechnologists are constructing new smart materials at an increasing pace. It is, however, important to realize that only a small part of the invented materials is applicable in medicine due to the limiting issues of toxicology and materials safety. Nanotoxicology has gained a lot of publicity, but the most serious safety issues are related to the technical use of nanoparticles and to the unintentional exposure to these materials [6]. Nanopharmaceuticals must be tested in preclinical toxicological tests and in clinical studies. Therefore, these products are not expected to have more

safety problems than regular pharmaceutical products after their acceptance for the clinical use.

In this chapter, we describe different nanotechnological systems for drug delivery and their potential applications for improved systemic and local bioavailability. The chapter is an overview that hopefully guides the interested reader to the more detailed texts on this issue.

22.2 Nanotechnological Systems in Drug Delivery

Nanotechnological systems have a particle size in the nanometer scale (1–1000 nm). In general, these systems can be divided into top-to-down and bottom-to-top systems. The first category includes nanosystems that are made by processing larger particles to smaller units. For example, drug nanocrystals are produced by milling larger particles to nanosize [4]. Bottom-to-top systems are constructed from molecules that adhere to each other thereby forming associated structures in the nanoscale. These systems are based on the principles of self-assembly and they have particular advantage of spontaneous formation and functional versatility.

22.2.1 Classification of the Technologies

The following paragraphs briefly describe the main categories of nanostructures that are relevant in drug delivery. The main points are compiled in Table 22.1.

22.2.1.1 Nanocrystals

Nanocrystals are small drug particles, usually about 100 nm in diameter, which are produced by milling drug particles in the presence of surfactant. Eventually, the

Table 22.1 Classification of nanotechnological drug delivery systems.

Class	Approximate size range (nm)	Applications	Clinical status
Nanocrystals	100–200	Dissolution enhancement	Accepted
Dendrimers	5–10	Drug targeting	Experimental
Nanoparticulates			
Liposomal	50–500	Intravenous drug delivery	Accepted
		Localized drug delivery	Experimental
Micelles	10	Drug delivery, solubilization (i.v.)	Accepted
Albumin	130	Intravenous drug delivery	Accepted
Polymeric	100–200	Drug delivery	Experimental
Peptide vesicles	10–100	Drug delivery	Experimental
Targeted nanoparticles	10–200	Site-specific drug delivery	Experimental
Nucleic acid complexes	50–200	DNA and siRNA delivery	Experimental

surfactant will cover the surface of the drug nanocrystals [4]. The main use of such particles is in the field of dissolution enhancement. Poorly soluble drugs (BCS class III and IV) show typically very slow dissolution, and, for that reason, they do not dissolve adequately during the passage of the tablet in the small intestine (typical transit time is 3 h). According to the law of Noyes and Whitney that is already more than 100 years old, increased surface area of the powder increases the rate of dissolution. From the bioavailability point of view, the increased dissolution rate is meaningless for most of the class I and class II compounds because their entire dose would dissolve anyway rapidly. In the case of class III and class IV compounds, the rate of drug dissolution can make a great difference. This was shown early in the case of digoxin when the importance of digoxin particle size on bioavailability was demonstrated [7]. There is indeed clear rationale for the use of nanocrystals in oral drug delivery.

22.2.1.2 **Self-Assembling Nanoparticulates**

Self-assembled nanoparticulates comprise various kinds of polymeric, peptide-based, and lipoidal systems. The rationale for their pharmaceutical use is to incorporate the drug into the system and thereby modify its solubility or delivery.

Self-assembled delivery systems form spontaneously in water solution when the structural component is added to water. Amphiphilic compounds form such structures and the features of the resulting nanostructures depend on the molecular properties of the amphiphile. Typically, these systems have a critical association concentration (e.g., critical micelle concentration (CMC)). Below this concentration, the compound exists as individual molecules (monomers) and orient toward the surface of water. Above the critical concentration self-associated structures are formed. The relative sizes of the polar head group and nonpolar chains determine the critical association concentration and morphology of the resulting structures. Micelles are formed by surfactants that have relatively large head groups compared to their nonpolar ends. Lamellar phases (i.e., liposomes) are formed when the both ends are of similar size. Tubular hexagonal phases result from the self-association of the molecules with small polar group relative to the wide hydrophobic end of the molecule. Lipid-like amphiphilic molecules can adopt various 3D structural orientations, which have been summarized earlier.

In addition to lipid-like molecules, polymers can also form self-assembling particulates [8]. As expected, the amphiphilic polymers do form polymeric micelles with polar part orienting towards the surface of the particle and the hydrophobic part orienting towards the core of the particle. Also, in this case, different complicated self-assembled structures can be tailored by using block copolymers with regular blocks of monomer units; but, only the simpler ones have been investigated so far in the context of drug delivery.

Biological molecules do show regularity at the level that is not obtained with synthetic polymers. Protein folding is a perfect example, but it is not yet well understood. Therefore, protein-based 3D drug delivery systems are difficult to design. However, small peptides with amphiphilic structure (e.g., V_6K, where six valines form hydrophobic part and lysine is the hydrophilic end group) can assemble

to form vesicles or tubes [9]. Peptides with regular repeating units may also self-assemble to fiber structures [10]. DNA, RNA, and oligonucleotides are versatile materials due to their ability to exactly recognize the complimentary sequences. Nanotechnologists have tailored even smiley-shaped DNA nanostructures. The self-assembling peptides and DNA-based nanostructures have only sparsely been explored in the field of drug delivery.

The size of the self-assembled amphiphilic structures varies from about 10 nm to a micron scale. Micelles are smaller than the vesicles, because they do not have internal aqueous core, and the wall is monolayer, not bilayer like in the liposomes.

22.2.1.3 **Processed Nanoparticulates**

Polymeric nanoparticles and nanocapsules are usually based on processing [11]. The processing may involve dispersion of the polymer solution in the dispersed organic phase in the continuous water phase and subsequent precipitation of the polymer by changes in the solvent composition. Nanoparticulates can be produced also by spraying techniques, including electrospraying [12]. The most commonly used materials include polylactide and polyglycolide and their copolymers. They are FDA-approved biodegradable materials with safe degradation products.

22.2.1.4 **Single-Molecule-Based Nanocarriers**

In the aforementioned cases, each particle contains typically at least thousands of molecules that are bound to each other by secondary chemical forces. The progress of chemistry since 1990s has provided a new class of materials, the dendrimers [13]. They are dendritic structures that are synthesized in generations around a core molecule that serves as a starting point. Large dendrimers may have even 10 generations, which means that it has 10 layers of dendritic structures in onion-like conformation. The simple dendrimers have spherical shape and they are much more monodisperse than most other synthetic polymers. Dendrimers are interesting materials for drug delivery purposes. They have been used for DNA and oligonucleotide delivery [14], but the dendritic shape as such does not provide improved properties compared to similar chemistry (poly-L-lysine) but linear or branched shape [15].

22.2.2
Pharmaceutical Properties of Nanotechnological Formulations

22.2.2.1 **Drug-Loading Capacity**

Drug-loading capacity of the system defines the dose of drug per individual particle. In principle, solid drug nanoparticle has the maximal loading capacity because it is nearly 100% drug. Drug nanocrystals have been mostly used for dissolution enhancement. In this context, the surface area per milligram of drug is the key parameter and this is defined by the particle size. For intracellular drug delivery and targeting, solid nanoparticles of pure drug have rarely been used. Abraxane is a paclitaxel product that is administered intravenously [16]. It contains drug crystals associated with albumin, but this is not a delivery system for intracellular drug

delivery, rather an approach to improve the drug dissolution after injection. The size of individual albumin molecules is 4–6 nm, whereas the paclitaxel-containing albumin nanoparticles are about 130 nm in diameter.

Vesicular systems can encapsulate both hydrophilic and hydrophobic drugs. They are localized either in the membrane (lipophilic) or in the aqueous core (hydrophilic) of the delivery system. In general, the micellar systems (surfactant or polymer based) are useful for the loading of hydrophobic drugs. Importantly, the size of the vesicular systems, such as liposomes, is in the range of 100–500 nm, and micellar systems are in the range of 5–10 nm. In terms of volume and drug-loading capacity per particle, this is a huge difference because the particle volume is proportional to the $(\text{radius})^3$ of the particle. Tenfold difference in the radius means thousandfold difference in the volume. Therefore, drug dose that is delivered per particle upon endocytosis is much bigger with larger particles than with the small micellar structures. However, the smaller micellar particles more easily gain access to the tissues because they can more easily extravasate from the blood circulation to the tissues.

Drug loading into nanoparticles can also depend on charge. For example, efficient loading of negatively charged nucleic acid-based drugs into the positively charged micelles, liposomes, or dissolved polymers is achieved by electrostatic binding [17]. This results in the formation of a new nanoparticle complex and disruption of the original liposomal or micellar structure.

22.2.2.2 **Processing**

The processing of the nanoparticulate structures is out of the scope of this chapter. It is important, however, to notice that in some cases the nanoparticulates may form spontaneously and drug is partitioned to the nanoparticulate structure by simple mixing. However, loading of hydrophilic drugs into liposomes requires reverse-phase evaporation or other processing methods, and likewise drug encapsulation into the polymeric nanoparticles often requires special processing. These factors depend on the drug and carrier properties and they are designed case by case.

22.2.2.3 **Biological Stability**

Biological stability of nanoparticles is an important determinant of their applications. The stability depends on the nanoparticle class.

In the case of self-assembling nanoparticulates, the critical association concentration affects their behavior. If the critical concentration of the amphiphile is high, the concentration may decrease after intravenous injection below the critical value thereby resulting in the dissociation of the particles [18]. This is the reason why surfactant-based micelles, with CMC in the millimolar range, are not useful as intravenous drug targeting vehicles as they do not retain their integrity long enough to enable improved target site bioavailability. Such micelles are, however, useful in solubilization of the drug to avoid the drug-induced irritation. If micellar drug solution is applied to extravascular site with limited dilution upon administration, the self-associated micelles may remain intact and enable localized drug delivery (e.g., in the skin).

Phospholipids have low CMC values in the nanomolar range. Therefore, this class of nanoparticulates is more suitable for intravenous administration, because the

phospholipid concentration remains above CMC even after i.v. injection. Thus, liposomal products are successful in the intravenous delivery of anticancer agents and the liposomes do not disintegrate in the blood circulation. Owing to their integrity, normal phospholipid liposomes are too stable for transdermal drug delivery. After their application on the skin, the liposomes stay on the skin surface and do not facilitate drug delivery across the skin. Only special classes of lipids, fusogenic hexagonal phase forming lipids such as DOPE or lysophospholipids, are able to fuse to the skin lipids and facilitate transdermal drug delivery by permeabilizing the skin barrier [19].

Polymeric micelles have critical association concentrations that can be modified by the polymer structures. The critical concentrations are lower than those in the case of surfactant-based micelles enabling the stability of the polymeric micelles in the circulation [18]. Polymeric micelles have been used successfully for drug targeting intravenously.

Typically, the processed polymeric nanoparticles are stable in the blood stream and elsewhere in the body [20]. Their degradation and drug release are determined by the chemical degradation rate of the polymer such as poly(lactide) and poly(glycolide). Such nanoparticles can be used for site-specific intracellular drug delivery if they have targeting moieties on the surface.

22.3 Delivery via Nanotechnologies

22.3.1 Delivery Aspects at Cellular Level

In principle, the nanotechnological systems could facilitate the overcoming of the biological barriers at tissue and cell levels.

Nanoparticulates may get across the *tissue barriers* depending on the type of the delivery system and tissue boundary (Figure 22.1). The size of the nanoparticulates is relatively large compared to the size of small molecular weight drugs. In tight epithelial and endothelial tissue linings, the size of the paracellular penetration pathway is about 2–3 nm [21]. This is clearly smaller than the size of the nanocarriers (10–1000 nm). Only some metallic nanoparticles, such as gold nanoparticles, can be in the size range that should allow paracellular penetration across tight epithelia. Current nanosystems do not fuse with the cell membranes either, and therefore, the only possible mechanism for crossing the tight epithelial and endothelial barriers is by transcytosis (Figure 22.1a) [22]. This process requires specific docking on the cell surface receptor, subsequent transcytosis, and release from the basolateral side of the membrane. Without specific cell biological mechanisms, the nanoparticulate systems are not useful in drug delivery across tight junction containing membranes, such as small intestinal wall, cornea, blood–brain barrier, and nasal epithelium.

There are several tissue boundaries in the body with more leaky character. For example, the vascular endothelium in the liver and spleen allows passage of even

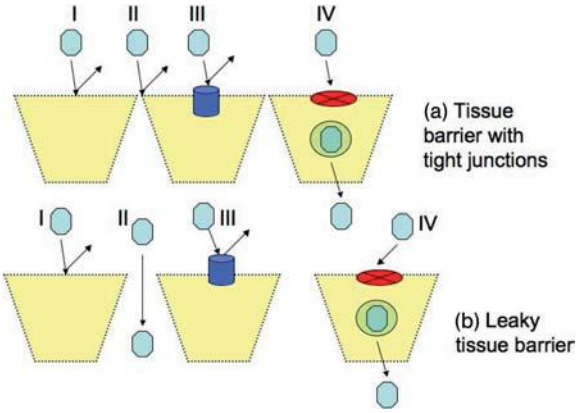

Figure 22.1 Nanoparticulate-mediated drug delivery across the tight (a) and leaky (b) tissue barriers. In the case of nanocrystals and drug solubilization systems, the dissolution rate is increased and free drug permeates across the tissue barrier with the appropriate mechanism. The nanoparticulate-bound drug behaves differently. The nanoparticulates are too large for the direct transcellular permeation across the cells' walls (I), for the paracellular diffusion through the tight tissue boundaries (a-II), and for the active transport by membrane transporters (III). The tight tissue boundaries include the intestinal wall, skin, cornea, conjunctiva, blood–brain barrier, placental barrier, and blood–retina barrier. In leaky tissue boundaries (e.g. fenestrated endothelia, sinusoidal vessels, and tissue boundaries disrupted by the disease states such as inflammation), the nanoparticulates may pass the barrier by paracellular permeation. In specific cases, receptor-mediated transcytosis may be possible (IV), but in this case specific recognition and transport mechanisms must be utilized.

micrometer-scale particles, and tumor vasculature can be extravasated by nanoparticulates of 200 nm and smaller (Figure 22.1b). In addition, many localized tissues such as vitreous in the eye or coronary vessel walls allow nanoparticulate diffusion after local administration.

In the case of *intracellular targeting* of the nanoparticulates, it is important to consider the intracellular target organelle, nanoparticle type, and characteristics of the cell (Figure 22.2). Nanoparticulates are simply too large to diffuse across cell membranes. They may, however, enter the cells via endocytic mechanisms [23]. These mechanisms involve binding of the nanoparticles to the cell surface and subsequent invagination of the cell membrane and formation of the endosomal vesicle (Figures 22.1 and 22.2). The size of the nanoparticle is important in this process. Only a few specialized professional phagocytic cells, such as macrophages and retinal pigment epithelium, are able to engulf large micrometer-sized particles [24]. Most cell types can endocytose only nanoparticles that are less than 200 nm in diameter. Endocytosis can be a receptor-mediated specific process, a nonspecific fluid-phase process, or an adherence-based process.

The endosomes can be further divided into clathrin-coated pits and caveolae [25]. The former is acidified and they deliver their contents to the lysosomes. Depending on the case, lysosomal delivery can be beneficial or vice versa. Nanosystems can be designed to release the drug resulting from the action of specific lysosomal enzymatic

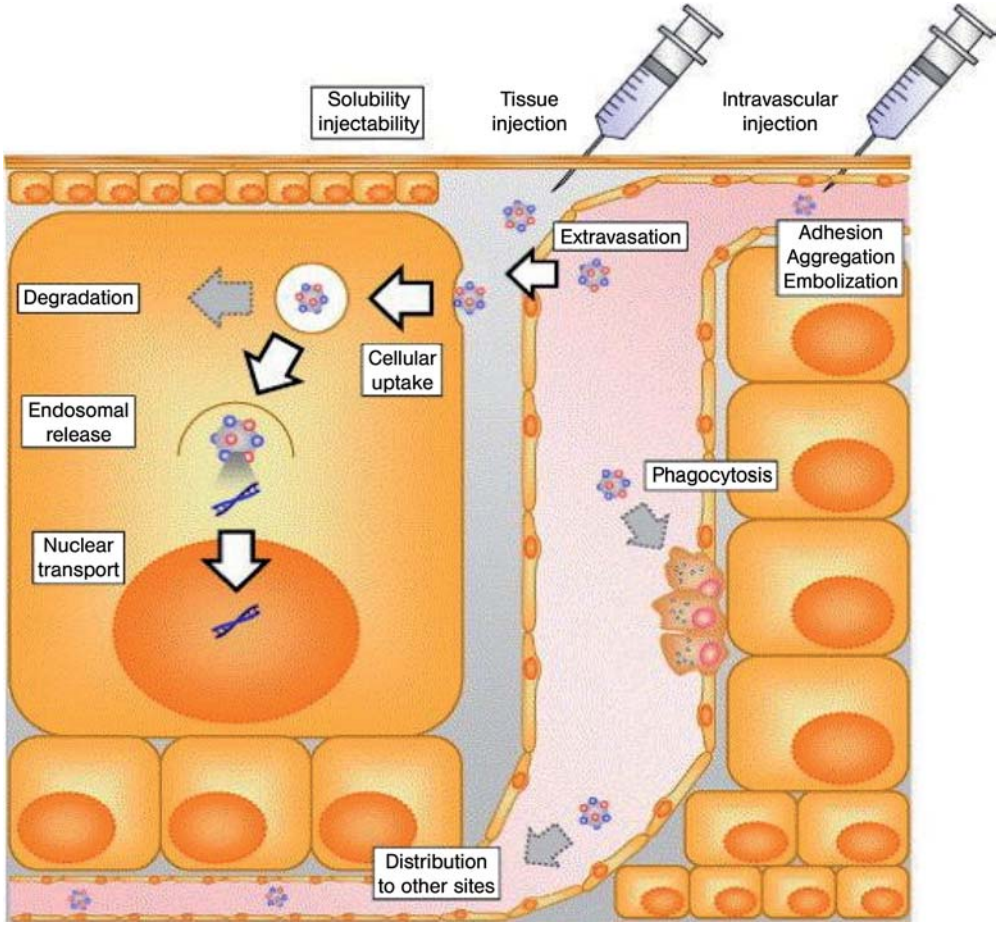

Figure 22.2 Fates of nanoparticulates after i.v. injection and local administration to the target tissue. After i.v. injection, the particles should avoid aggregation and embolization to avoid entrapment into the lung capillaries. Kuppfer cells of the liver phagocytose major part of the nanoparticulate dose (illustrated on the right), but this may be slowed down by nanoparticulate surface modifications. In the target tissue (on the left), the nanosystem should enter the vascular endothelial cells, if they are the target cells, like in the case of neovascularization. Otherwise, they should escape from the vasculature, dock to the target cell surface, and internalize into the target cells by endocytosis. Depending on the case, the intracellular target may be cytosolic, lysosomal, nuclear, or elsewhere. With the exception of the lysosomal targets, the nanoparticulates should escape from the endosomes and enter the cytoplasm (e.g., siRNA) or nucleus (e.g., DNA). The nanoparticulates may be injected or given directly to the tissue of interest. Then, the barriers of Kupffer cells and vascular walls are avoided. The issues of cellular uptake and intracellular kinetics are relevant also in this case. This figure is taken from Ref. [43].

cleavage. In this case, the drug as such should be resistant to the acidic lysosomal pH and the catalytic activity in this organelle. In many cases, the lysosomal delivery should be avoided, and the drug ought to escape from the endosomes to the cytoplasm before entering the lysosomes. The escape can be facilitated with special

design of the nanoparticulates. For example, the acidification in the endosomes (from pH 7.4 to 5.5) and reducing environment can be utilized as triggering mechanisms that allow nanoparticulate activation and endosomal escape [26]. Membrane-active peptides, pH-sensitive lipids, and reducing polymer structures with disulfide bridges have been utilized as nanoparticulate components for this purpose.

Cytoplasm is an important target for siRNA and antisense oligonucleotides whereas transcription factors and plasmid DNA should be delivered into the nucleus (Figure 22.2). Cytoplasm is a highly viscous medium where passive diffusion of nanoparticles, and macromolecules are very slow [27]. It is very appealing to search for the means by which the nanoparticulate transport in the cytoplasm and delivery into the nucleus can be maximized [28]. Specific nuclear localizing peptides have been attached to the nanoparticulates for the nuclear delivery but their efficacy is still not adequate [29].

22.3.2
Nanosystems for Improved Oral Drug Bioavailability

The steps in oral drug absorption have been described in detail elsewhere in this book. The preceding discussion about the cellular interactions of the nanoparticulates suggests that the nanoparticles are not physically optimal for drug delivery across the relatively tight intestinal wall (Figure 22.1). Rather, other mechanisms are more viable. *First*, the nanoparticulates can be used to improve drug dissolution especially in the case of BCS class III and class IV compounds. This can be accomplished by making pure drug nanocrystals, as discussed above. Another alternative is to use self-assembling structures, such as self-emulsifying systems, to solubilize the poorly soluble drugs [30]. These techniques have shown some improvement in systemic bioavailability after oral drug administration. *Second*, the retention time of the particles in the intestine can be prolonged by adhering the particles to the gut wall. This approach involves the use of lectin moieties at the particle surface. Lectin binds to carbohydrates on the gut wall and generates higher localized drug concentration next to the intestinal wall thereby increasing drug absorption [31]. *Third*, the transcytosis mechanism can be used [22]. As far as the third approach is concerned, only vitamin B-12 utilizes transcytosis in its absorption [32]; otherwise, this approach has not been successful. It is difficult to obtain high enough permeation through the intestinal wall with this mechanism.

It is important to realize that in the first and second option, the local concentration of the free drug is increased by the nanosystems. The drug may be absorbed by passive diffusion or active transport, but it is not specifically carried by the nanoparticulates.

22.3.3
Nanosystems for Improved Local Drug Bioavailability

Improved local tissue-specific bioavailability can be reached either by systemic administration intravenously or by localized direct injection in the vicinity of the target tissue (Figure 22.2).

The local administration of the nanoparticles can be used either to increase the retention at the site of administration or to control the drug release. Intravitreal drug administration into the eye is an interesting example. If a small molecular weight drug is administered in the form of water solution to the vitreous, the concentration decreases rapidly, because the drug diffuses to the systemic circulation across the blood–retina barrier or via the anterior chamber [33]. Therefore, the drug concentration profile in the vitreous shows rapid decline after initial high concentration peak. When the drug is administered in the liposomes, prolonged concentration profile is obtained due to the hindered permeation of the liposomes across the barriers. Thus, nanoparticulates modulate the concentration profile of the free drug in the vitreous by removing the high-peak concentration and by prolonging the retention in the vitreous. Similar principles are applicable to many other sites of localized injections: nanoparticulates increase drug retention at the site of injection and modify the concentration profile toward controlled release profile (e.g., at the surgical sites).

If cell-specific targeting is sought, the nanoparticle should bear appropriate ligands on its surface to recognize the cell of interest. This was exemplified by recent study with nanoparticulates that recognize CD44 receptors responsible for internalization of hyaluronic acid-coated DNA complexes [34]. Lipid–DNA nanoparticulates were also able to transfect corneal epithelial cell surface that released the encoded protein to the basolateral side of the epithelium [35]. As such, the protein would not diffuse through the tight junctions on the corneal epithelial surface (Figure 22.3). This

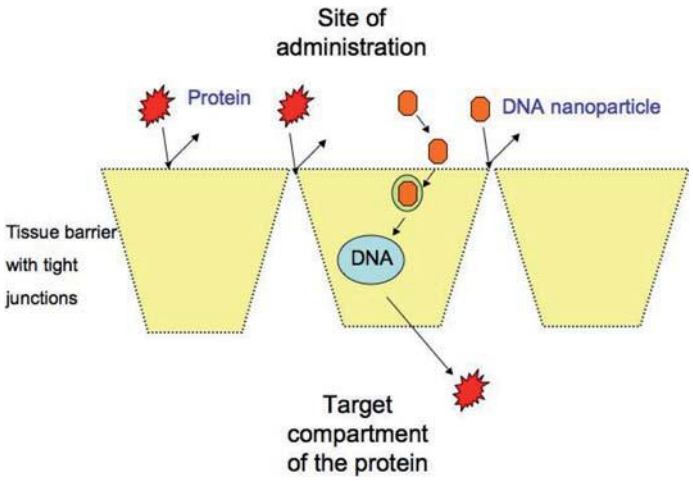

Figure 22.3 Epithelial cells can be transfected to serve as secreting platform of the therapeutic protein. Protein as such does not permeate across the tissue boundary. The nanoparticulates that encode the protein also cannot permeate the epithelial barrier, but they can internalize to the outer most all layer of the epithelium. These cells are transfected and subsequently secrete the therapeutic protein to the basolateral target compartment. This principle of circumventing the apical tight junctions was demonstrated recently in the eye [35]. DNA nanoparticulates were administered to the tear side of the cornea and the transgene product, SEAP, was secreted to the anterior chamber of the eye.

method circumvents the tight epithelial barrier by transfecting the surface cells to secrete the therapeutic protein to the other side of the barrier.

The systemic i.v. administration can be used for targeted localized drug delivery with nanosystems (Figure 22.2). This is particularly appealing in the case of cancer medications, because these drugs cause serious adverse effects at therapeutic doses [36]. In addition, the metastases are difficult to treat with local injections. Therefore, most nanoparticulate studies dealing with systemic administration are directed to the cancer treatments. This approach is not an easy one, because a major fraction of the nanoparticulate dose is captured by Kuppfer cells in the liver (Figure 22.2). The half-life of the particles in the circulation is increased by the "stealth" coating of the particles. Polyethylene glycol (PEG) moieties on the nanoparticulate (polymeric micelle, liposome, polymeric nanoparticle, and DNA complexes) surface prevent the adherence of plasma proteins on the surface. Therefore, the Kuppfer cells do not recognize these particles and long retention times in plasma (1–2 days) are achieved. Eventually, a large fraction of these particles ends up in the liver as well, albeit at a slower rate. The particles must extravasate from the blood circulation, and PEG-coated nanosystems have a higher probability of extravasation in the tumors due to their leaky vasculature. Thereafter, the cell-level issues (see above) determine the fate and therapeutic efficacy of the nanoparticulate systems (Figure 22.2).

Systemic administration of drugs in the form of nanoparticulates intravenously has been widely studied. Currently, there are some liposomal anticancer and antifungal medications in clinical use. These products show relative increase in drug bioavailability in the target sites. They are not, however, active targeting systems with recognition ligands on the surface.

22.4
Key Issues and Future Prospects

The delivery of drugs to difficult-to-reach targets, such as brain and tumors, remains a major challenge. Biotechnological drugs, such as gene medicines and some proteins, need improved nanotechnological formulations for their intracellular delivery. Design of such delivery systems requires interplay between the delivery system and the biological machinery to reach the therapeutic goals. Viruses have evolved to use the cells for their own purposes and in doing so they deliver their genetic cargo into the target cells in elegant ways. Successful functional mimicking of biological self-assembling nanostructures is essential to the progress in this field.

Another important future issue in the field of drug delivery nanotechnology is the use of smart responsive materials and small devices based on such materials. The materials may take into account the human physiology by releasing the drug at needed rate at the right time. These systems are also applicable in the design, fabrication, and use of advanced nanobiosystems for cellular integration and tissue engineering. The opportunities exist for the use of functional biomaterials and therapeutic drug targeting and delivery systems that combine both biological and

engineering aspects of drug delivery [37], like in the case of the off-water fabrication and surface modification device consisting of asymmetric 3D SU-8 microparticles for drug delivery and tissue engineering [38].

Nanoparticulate systems can be designed to bear multiple components and functions, including activation at the target site or by external signal (e.g., light or magnetic field) [39, 40]. Such future trends would also involve the use of new types of nanomaterials, such as carbon nanotubes [41] and functionalized metallic nanoparticles [42]. Taking the safety issues into account is an imperative, as many nanomaterials for the technical purposes do not meet the criteria that are required for pharmaceutical materials. Anyway, it is likely that the current research investments in the fields of nanomedicine and pharmaceutical nanotechnology will lead to improved drug therapies by improving the drug bioavailability at the target site.

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