Chapter 1
Targeted Drug Delivery Systems: Strategies and Challenges

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Abbreviations

DDS	Drug delivery system
TDDS	Targeted drug delivery system
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
BBB	Blood–brain barrier
RES	Reticuloendothelial system
PEG	Poly(ethylene) glycol
MNP	Magnetic nanoparticles
SPION	Superparamagnetic iron oxide nanoparticles
ADEPT	Antibody-directed enzyme prodrug therapy
GDEPT	Gene-directed enzyme prodrug therapy
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
HSV	Herpes simplex virus
EPR	Enhanced permeability and retention
SLN	Solid lipid nanoparticle
FDA	Food and Drug Administration
siRNA	Small inhibiting RNA
RNAi	RNA interference
TNF-α	Tumor necrosis factor alpha
WHO	World Health Organization

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TB	Tuberculosis
MSNP	Mesoporous silica nanoparticles
PEI	Polyethyleneimine
CNS	Central nervous system
RBC	Red blood cells
PLA	Poly (D,L-lactide)
HAART	Highly active antiretroviral therapy
MDR	Multidrug resistance
AZT	Azidothymidine
BCSFB	Blood–cerebrospinal fluid barrier
CMT	Carrier-mediated transport
RME	Receptor-mediated endocytosis
AME	Absorptive-mediated endocytosis
APO E	Apolipoprotein E
LDL	Low density lipoprotein
CSSS	Cyanoacrylate skin surface stripping
PLGA	Poly (DL-lactide-co-glycolide)
BRB	Blood–retinal barrier
RPE	Retinal pigmented epithelium
P-gp	P-glycoprotein
PepT	Peptide transporters
IBD	Inflammatory bowel diseases
IBS	Irritable bowel syndrome
CDDS	Colon targeted drug delivery systems
GIT	Gastrointestinal tract
RISC	RNA-induced silencing complex
CPPs	Cell penetrating peptides

1.1 Introduction

In this complex and ever-evolving world of medicine it has become increasingly important to address the issues of the drug development involving the delivery of specific drugs to their site of action in therapeutically acceptable doses. With the advancement of the pharmaceutical sciences, the industry has certainly observed discovery of several new drug molecules ranging from small molecule drugs to macromolecules like proteins and peptides; but the ultimate goal of achieving disease-free conditions in the patients is often left hanging due to several hurdles relating to physicochemical and molecular intricacies of the "free" drugs and unapproachability and under-dosing of most of the biological/pathological targets. To improve on these situations drug delivery systems (DDS) are employed which could either be a formulation or a device that facilitate the administration of a drug to the body whilst improving its pharmacokinetic and biodistribution profiles and the efficacy and safety of the whole treatment. Targeting the drugs (and DDS) involves the improvement of the specificity of the system towards the pharmacologically relevant target in

the body. Targeted drug delivery systems (TDDS) involve the administration of the DDS to the patient, delivery of the DDS at the target (pathological) site, release of the active ingredients in/around the target, and avoiding nonspecific toxicity in normal cells. The above concept of targeted drugs—magic bullet—was first conceived by Paul Ehrlich in early twentieth century and over the past decades several strategies have developed to achieve targeting [1, 2].

A TDDS can be broadly understood as a system that carries out the following functions:

- Facilitate the therapeutic substance to reach the site of action from the site of administration where the target site can be organ, tissue, cell or even specific cell organelles.
- Release the therapeutic payload in its active form in/around the target site presenting effective therapeutic levels at the site of action.
- Protect the drug/gene from the detrimental effects of environmental factors such as pH, enzymes, etc.
- Avoid toxicity or adverse reactions of the drug/gene on nonspecific normal cells and facilitating administration of lower doses to achieve therapeutic/diagnostic benefits.

Research in the field of targeted drug delivery has given several options of carrying out the above functions:

- Direct targeting to site of action, e.g., topical applications for skin diseases.
- Use of external stimuli, e.g., ultrasound.
- Chemical modification of the drug to make its physicochemical properties ideal for the delivery which includes prodrug approach of attaching a promoiety to the drug.
- Use of nanocarriers like liposomes, polymeric micelles, polymeric nanoparticles, solid lipid nanoparticles which can be functionalized further with attachment of targeting ligands, antibodies.

An efficient TDDS ideally should possess the following properties:

- The drug-conjugate/drug-carrier should reach the intended site of action (organ/tissue/cell/cell organelles) with minimal nonspecific accumulations.
- The chemical conjugation or physical encapsulation of the drug/gene with the targeting ligands or carriers should not inactivate or alter the drug/gene action on the intended site of action; the TDDS should be able to protect the drug from environmental factors such as enzymatic degradation till they reach the target.
- The chemical conjugation or physical encapsulation of the drug/gene with the targeting ligands or carriers should not inactivate or alter the ligand or carrier activity and function to reach the intended site of action.

This chapter has been divided into sections which cover the general strategies of developing TDDS, the use of TDDS in diseases like cancer, HIV/AIDS, tuberculosis, and the use of TDDS to target specific organs and locations. While the targeted drug delivery systems require in-depth study on their own, the intentions of this chapter remain to provide only an overview of the relevant challenges and strategies.

1.2 Targeted Drug Delivery: General Concepts

Targeted drug delivery at the site of action can be carried out by direct techniques usually involving invasiveness: direct injection, catheter [3, 4], gene-gun [5], etc. Though these systems show direct delivery, invasiveness is not patient convenient and expensive to carry out in many cases. As a result, efforts are put into developing TDDS which involve chemical, physical and biological modifications with or without the use of carriers.

Changes done to improve targeting the drug include study of structure activity relationships to improve the physicochemical properties for targeting. Small-molecule drugs intended for brain delivery unable to penetrate the blood–brain barrier (BBB) may be made more lipophilic to aid penetration through the BBB, provided they have small size. Prodrugs can be made to improve the pharmacokinetics of the drugs. Small molecule drugs are chemically modified by attaching "promoeities" rendered pharmacologically inactive and are metabolically activated in vivo into active drugs only after reaching their intended target [6]. Drugs may be conjugated with antibodies, peptides, aptamers, folic acid, etc. to generate targeted prodrugs [7].

On the other hand, the drugs can be incorporated into nanocarriers or nanosystems. These include drug carrier systems like liposomes, polymeric micelles, polymeric nanoparticles, polymer–drug conjugates, nanogels, carbon nanotubes, etc. [8]. The nanosystems are a very efficient way to deliver the drugs or genes. The major advantage of using such systems is that the pharmacokinetic behavior of the drug-loaded nanocarriers depends on the nanosystems rather than the drugs or genes, which makes it easy to control with the help of further targeting. The nanoparticles described in this chapter are <300 nm, unless noted otherwise.

Such drugs/drug carrier systems depend on a few modes of targeting which are broadly classified into passive and active targeting.

1.2.1 Passive (Physiology-Based) Targeting

Passive targeting is present naturally in the human body. Hormones, neurotransmitters, growth factors, etc. have a natural tendency to go and target the receptors at their sites of action, e.g., insulin and insulin receptors. This concept can be applied to the drugs too. The accrual of drugs/drug-carrier systems at the intended site of action by the action of physicochemical and physiological factors is passive targeting [9].

Certain tissues under diseased conditions present opportunities in terms of modified physiologies which can be exploited by passively targeting nanocarriers. A presence of leaky vasculature with large gaps in the blood vessel's epithelial layers has been observed in cases of inflamed tissues in inflammatory bowel disease and inflammatory rheumatoid arthritis [10] and in tumor tissues [11] which make it possible to passively target the administered nanocarriers of appropriate sizes to extravasate into the target tissue. Although the tumor tissue has limited lymphatic drainage [12] and the inflammatory tissues have a functioning lymphatic drainage,

the passive targeting can still benefit the inflammatory diseases. Accumulation of nanocarriers is also observed in the liver due to large fenestrations in it and this can be used for liver targeting in liver diseases. This phenomenon wherein the nanocarriers accumulate into the diseased tissues because of loose fenestrations and/or poorly formed lymphatic drainage is termed as enhanced permeability and retention (EPR) effect [11, 13].

The nanocarriers are largely affected to clearance by the reticulo-endothelial system (RES) comprising of macrophages and mononuclear phagocytes. This fact can be used to passively target the macrophages and even lymph nodes and spleen to treat infections that affect the RES (e.g., leishmaniasis and malaria) [14].

Often modifications (e.g., attachment of polyethylene glycol; PEG) are made on nanocarriers to make them long-circulating, avoiding the RES and granting them time to accumulate at target sites in high amounts (long-circulating nanocarriers) [15].

Passive targeting also benefits from the presence of internal stimuli, such as pH difference (e.g., low pH in tumor microenvironment [16]), redox systems (e.g., exploiting high glutathione in cancer [17]), etc. in the diseased tissues. Stimuli-sensitive drug targeting systems will be spurred by such stimuli to release the drug only at the target site and spare the normal tissues. Such stimuli-responsive systems have been extensively studied [18–22].

1.2.2 Active Targeting

While significant results have been observed with passive targeting, the pursuit of better control on accurate drug delivery has led to a lot of research in active targeting methods. Appropriate modifications and functionalization on the drugs or drug carriers afford them affinity towards specific receptors/markers on cells, tissues or organs. Factors such as the disease, the intended target organ, and a larger presence of targetable components on the target organ/cell (e.g., transferrin receptors in tumor) than in normal cells are taken into consideration while deciding on the targeting moiety to be attached to the therapeutic substances. Modifications on the drugs or drug carriers can involve the use of ligands such as peptides, antibodies, sugars, lectins, etc. Thus, on administration to the body, the targeting moieties will enable the drug/drug-carriers to efficiently reach only the intended sites of action and avoid nonspecific accumulations and related side effects.

Apart from the administration of such actively targeted systems, there are technologies available to further control the delivery system which are covered below.

1.2.2.1 Targeting Mediated by External Stimuli

External stimulus, such as magnetic fields and ultrasound, acting on on nanocarriers, are employed to perform imaging, to target and release drugs from the nanocarriers at the intended site of action. The benefits of using this mode of active

targeting are: real-time targeting, targeting deep-seated tissues, simultaneous imaging and therapy. The approach can also combine different external stimuli, for example, ultrasound and magnetic field, for enhanced targeting and efficiency.

The use of magnetic nanoparticles (MNP) as imaging agents for magnetic resonance imaging, magnetic drug targeting and hyperthermia treatment is well explored in the field of targeted drug delivery. The MNP can be either metallic or bimetallic or superparamagnetic iron oxide nanoparticles (SPION); among them SPION are widely studied for biomedical applications because of nontoxic nature, ability to be functionalized with different targeting coatings and can encapsulate drugs in reasonable quantity. Optimizing the MNP as well as the external magnet is of prime importance because on application of magnetic field, they must be able to generate enough magnetic moments and magnetic gradient that the MNP can overcome the force of the blood-flow (rating from 0.05–50 cm/s) depending on the target area [23, 24]. The MNP have found several uses in thrombolytic therapy [25, 26], intravascular imaging and cardiovascular diseases [27–29], tumor imaging and treatment [30–35], as well as delivery across the blood–brain barrier [36, 37].

Ultrasound has been used previously for contrast imaging, and it is explored at length for use in drug delivery. Ultrasound mediated targeting can lead to disruption of the drug-loaded carriers (microbubbles, micelles, etc.) causing drug release; exact mechanism of release is still under study. Furthermore, ultrasound focusing has also been found to cause reversible disruptions (Fig. 1.1) in the intravascular endothelial layers creating pores for the drug to enter the extracellular space of the target tissue. This occurrence was also observed with blood–brain barrier/blood–tumor

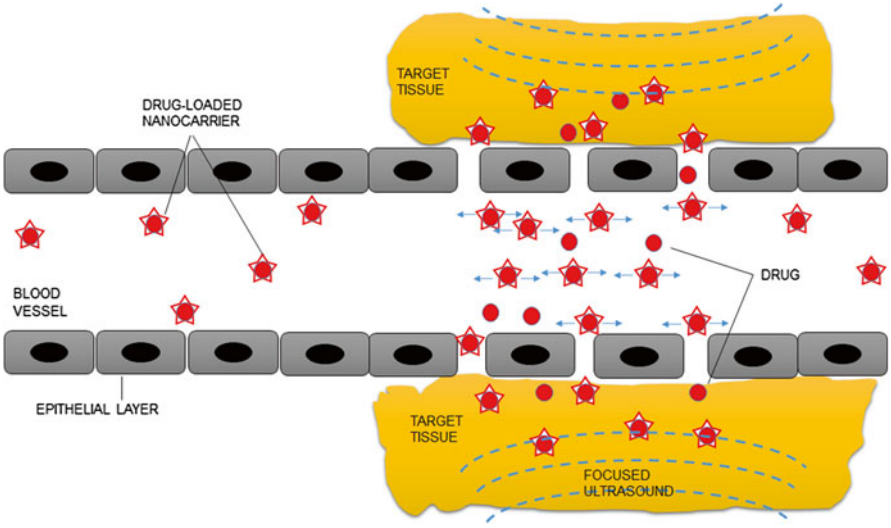

Fig. 1.1 External stimulus of focused ultrasound leads to (**a**) reversible disruptions and gaps in the epithelial cell layer allowing drugs/drug nanocarriers to escape the blood vessels into the target tissues, (**b**) disruptions to the nanocarriers to release the drugs around the target tissue

barrier [38–40]. Another benefit of using the ultrasound-mediated delivery is in targeted hyperthermia when combined with temperature-sensitive nanocarriers [41]. The generation of hyperthermia can prove cytotoxic for nearby tissues and in case of tumor treatment this is highly beneficial; the hyperthermia will kill tumor periphery cells and open the way for simultaneously administered drugs to enter the core of tumor tissue for enhanced killing. Studies with ultrasound therapy find applications in tumor imaging [42], tumor treatment [43–48], thrombosis [49, 50], cardiovascular diseases [51]. This mode of targeting also gives the opportunity to image and trigger release of the drug at the same time [52–54], and also to combine it with magnetic field applications for enhanced benefits [55, 56].

1.2.2.2 Antibody-Directed Enzyme Prodrug Therapy (ADEPT)

This approach is a two-step approach: (1) an activating enzyme is specifically delivered to intended site of action with a targeting antibody (e.g., tumor-specific antibody, anti-TAG-72) (2) a subsequent administration of substrate prodrug. The advantage with such a system is a single enzyme at the target site can activate multiple prodrugs and increase the load at the target site. A 3-phase ADEPT was performed in human ovarian carcinoma xenografts in mice. First, an enzyme–antibody complex—AB57-F(ab′)$_2$-CPG2—was allowed to localize in the tumor followed by a wash step of removing blood CPG2 by anti-CPG2 antibody administration to avoid nonspecific activation of the prodrug. Lastly, a benzoic acid mustard-derived prodrug was injected leading to tumor growth delay [57].

A modification of this approach is the *gene-directed enzyme prodrug therapy* (GDEPT). Here, instead of antibody-targeted enzyme, a gene is targeted to the intended site where it transcribes and translates to produce enzyme intracellularly which acts on the subsequently administered prodrug [7].

1.2.2.3 Targeting in Gene Therapy

This section primarily discusses about the strategies required to transport genes into their required site of action which is either the cytoplasm or the nucleus. Gene gun as mentioned previously is a physical method to directly transfer DNA and RNA with high transfection efficiency but because it is invasive and requires special setup it is not widely preferred. The current gene therapy employs viral and nonviral vectors.

The viruses have a unique ability to transfer their genes into cells. This function can be utilized to deliver genes. The bottom line, though, is that the viral vectors have to be modified to be devoid of virulent pathogenesis and replicative genes. The commonly used viral vectors include adenovirus, baculovirus, herpes simplex virus type 1 (HSV-1), etc. [58–60]. Utmost care has to be taken when designing these systems because the viral vectors are notorious for adverse effects such as inflammatory and immune responses, activation of latent infections, incorporation of transgenes into the host genomes and permanent expression persistence.

Nonviral methods to target gene transfer are not proficient with transfection efficiency but they are generally safer than the viral vectors. Polymeric micelles, liposomes and other nanocarriers have been studied to deliver the nucleic acids [61–63].

Thus presented are the classifications and the strategies of targeted drug/gene delivery systems. The next few sections feature the benefits of TDDS in various diseases.

1.3 Targeted Drug Delivery: Disease-Based Strategies

1.3.1 Cancer Specific Strategies

Cancer is uncontrolled growth of abnormal cells characterized by mutations which help the cells to proliferate, avoid apoptosis and develop survival proteins. Perhaps, cancer is the disease on which the most research in targeted drug delivery is focused and for a good reason. Even though chemotherapy, surgery and radiotherapy are available, the cancer manages to remit and regrow in most of the cases. It is a disease where the survival of patients is measured in weeks and months only even if some of the most advanced drugs are used. There are several reasons why cancer is so difficult to treat.

To begin with, the tumor microenvironment (higher interstitial fluid pressure, low extracellular pH, and formation of irregular tumor vasculature) as well as the cellular level (over-expression of efflux transporters, defective apoptotic machineries, and altered molecular targets) attribute multidrug resistance (MDR) towards the drugs [64]. Second, most of the chemotherapeutic drugs do not possess desirable physicochemical and pharmacokinetic properties. They have low solubility and stability, are highly nonspecific in nature and show high toxicities and are inconvenient to the patients. Therefore, targeted therapy in cancer plays a very important role. Nanopreparations are primarily employed to achieve this task [15] and approved products include Myocet® (liposomal doxorubicin), Daunoxome® (liposomal daunorubicin), Doxil® (liposomal doxorubicin), Depocyt® (liposomal cytarabine), Oncaspar® (monomethoxypolyethylene glycolol conjugation to L-asparaginase), Genexol-PM® (paclitaxel-loaded polymeric micelle), Abraxane® (albumin-bound paclitaxel particles), etc. Apart from these there are several other studies in various stages of clinical trials. Antibodies directed towards cancer therapy include Rituxan® (rituximab), Herceptin® (trastuzumab), Campath® (alemtuzumab), etc.

Methods other than using nanopreparations have also been examined. A recent study used chimeric antigen receptor-modified T cells targeted towards the CD19 expressing chronic lymphocytic leukemia (CLL). This research was performed in two children with complete remission of cancer with one having a relapse. The researchers have called for further work but the underlined principle exhibits the benefits of lentiviral-vector targeting [65].

The nanocarriers can target the tumor via passive targeting and active targeting. As explained previously, passive targeting involves the physicochemical properties of nanocarriers and the physiological factors. The tumors are rapidly proliferating

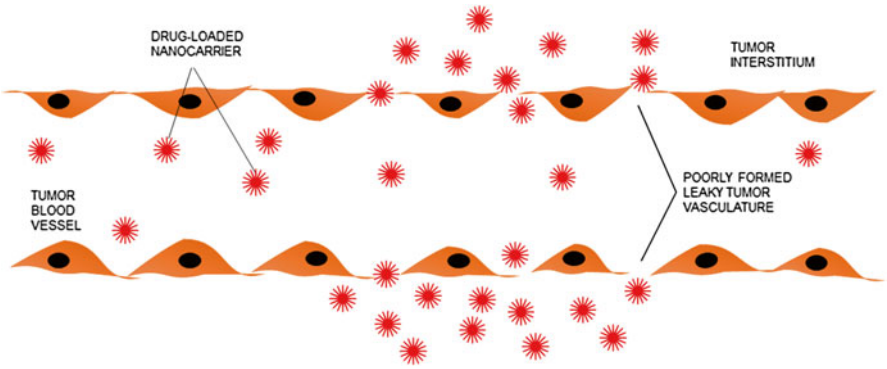

Fig. 1.2 Enhanced Permeability and Retention (EPR)—Poorly formed tumor vasculature with leaky vessels and meager lymphatic drainage can be exploited by passively targeting the nanocarriers up to size of 500 nm

cells and they require nutrients in large amounts. As a result, the tumors expedite the blood vessel formation leading to irregular and leaky tumor vasculature. The gaps in the endothelial layers allow the nanoparticles up to 500 nm in size to extravasate into the tumor tissue (Fig. 1.2). The absence of a proper lymphatic drainage helps to accumulate the drug-loaded nanoparticles in the tumor tissue. EPR is responsible for passive targeting in tumors [13, 66]. To enhance this effect the nanopreparations are modified by motifs like polyethylene glycol (PEG). In effect the presence of PEG modifications help nanocarriers avoid RES uptake and they circulate longer in the blood allowing them more time to accumulate in the tumor tissue.

Active targeting of the nanopreparations involve modifications that will not only help target the tumor tissue but also overcome the resistance factors. For instance, ATP-binding cassette transporters like P-glycoprotein on the cell membrane are responsible for efflux of several drugs and generating multidrug resistance [64]. To bypass such efflux transporters, drugs have been administered in nanocarriers with improved tumor inhibition efficiencies [67, 68]. Nanocarriers can also be actively targeted by conjugation with targeting ligands such as transferrin, folate, antibodies, etc. [69–73]. Interesting strategies to target tumor also involve stimuli-responsive nanoparticles which may even be dual targeted to facilitate step-by-step entry into the tumor cells [18, 74–76]. Several other examples of targeting various facets of cancer are discussed in these reviews [64, 77, 78]. We are not discussing a huge area of tumor targeting in details, since a broad variety of recent publications exist on this subject [79–83].

1.3.2 Targeted Drug Delivery Towards Infections

Infections by bacteria, fungi, virus and other microorganisms were the cause of widespread diseases and fatalities throughout the world in the early part of the twentieth century. As the understanding of the pathology expanded and the

discovery of antibacterial penicillin came through, the number of antimicrobial and antiviral agents have been developed. Essentially the antimicrobial inhibit or interrupt important cell cycle processes of the microorganisms and kill them. Even though we have been able to reduce the huge number of fatalities due to preventive and therapeutic actions, the menace of infections is still present in several developing and underdeveloped nations. Most of such countries are unable to meet the demands of antimicrobials to control infections such as malaria, tuberculosis (TB), HIV/AIDS, cholera and many more. As a result there is still a prevalence of such diseases. Moreover, it has now been observed that the unchecked abuse of antibacterial agents have resulted in developing resistant strains of the once sensitive pathogens [84]. While new antibiotics (oritavancin, telavancin, etc.) are in the various stages of drug discovery and clinical trials, their development will take time to reach the market [85, 86]. This situation demands for TDDS for the current class of drugs.

A lot of interest has been generated to develop TDDS especially the ones using nanoparticles, for infectious diseases, with their simplest advantages being the ability to deliver a variety of drugs and even genes, the ability to give sustained and targeted delivery while avoiding the toxicity as well as the ability to deliver combination of multiple drugs at the same time to overcome drug resistance. Liposomes, SLN, polymeric nanoparticles and other forms of nanoparticles are frequently studied for the TDDS development [87]. An FDA approved injectable liposomal formulation for amphotericin B, AmBisome (Gilead Sciences, Foster City, California, USA) is employed for the use against several infections. A targeted drug delivery for the AmBisome was performed in mice models of disseminated aspergillosis, and it was observed that the liposomal formulation was superior in efficacy compared to the amphotericin B deoxycholate in sterile water [88].

Techniques similar to observed in targeted cancer treatment, ligands, antibodies, peptides, etc. can be attached to the nanocarriers to enhance the efficiency of the anti-infectious treatments. The cell walls of *Helicobacter pylori* have carbohydrate receptors to which lectin-conjugated nanoparticles could specifically bind. Thus, a targeted preparation of lectin-conjugated gliadin nanoparticles was studied for receptor-mediated targeting towards *H. pylori* [89]. Studies have also been undertaken to deliver genes to infected cells. A TDDS for dengue virus infection was recently developed to deliver siRNA in a novel manner. The Dengue virus is known to affect the human dendritic cells as well as macrophages. Hence, a dendritic cell-targeting 12-mer peptide (DC3) was fused with nona-D-arginine (9dR) residues to form a DC3-9dR-mediated delivery of siRNA targeting the tumor necrosis factor alpha (TNF-α) which was able to target the dendritic cells as well as deliver the siRNA effectively; the result of which was suppressed virus replication and virus-related symptoms [90].

The remaining part of this section is an overview of TDDS developed for specific infections.

1.3.2.1 Tuberculosis (TB)

According to World health Organization (WHO), *Mycobacterium tuberculosis*, the bacteria responsible for tuberculosis, has caused infection in 8.6 million people worldwide by 2012. The infection is transmitted through air and it deposits in the alveolar region of the lungs. Current therapy employs antibiotics like rifampicin, isoniazid, ethionamide, etc. and often combination therapy is prescribed to attack the *M. tuberculosis*. Still, the bacteria has developed resistance against most of the drugs and when the condition is aggravated by concomitant HIV/AIDS presence, patients usually do not survive the infection well [91, 92]. Also, the drugs present serious side effects such as hepatotoxicity [93].

Hence, research is undertaken to develop targeted drug delivery for TB. Since the bacteria are taken up by the macrophages/monocytes in the lungs, the opportunity for targeting receptors such as lectin (mannose) receptors, immunoglobulin receptors, complement receptors, etc. expressed on such alveolar macrophages is presented [94]. Moreover, the macrophages have an innate response of phagocytizing the nanoparticles, hence targeting them makes a valid choice for treatment of TB. Most of the TDDS developed for TB prefer the pulmonary route of administration because the TB infection is primarily localized in the lungs.

Active targeting of the lectin receptor on the alveolar macrophages of rats via pulmonary administration of mannose-coated liposomes containing ciprofloxacin was performed giving increased drug uptake in the lungs as compared to the free drug; the plasma concentration of ciprofloxacin was also low with the targeted liposomes in comparison to the free drug exhibiting the benefits of using the targeting strategy [95]. A similar study was carried out via intratracheal administration of different concentrations of mannose in mannose-coated liposomes resulting in selective targeting and increased uptake in the alveolar macrophages of the rats [96]. Pandey and Khuller developed nebulized solid lipid particles incorporating a combination of rifampicin, isoniazid, and pyrazinamide for bronchoalveolar drug delivery in *M. tuberculosis* infected guinea pigs. As compared to 46 daily doses of orally administered drugs, the nebulized formulation achieved a complete removal of the tubercle bacilli from lungs and spleen after just seven doses of administration, each dose administered periodically on every seventh day. Moreover, hepatotoxicity was not observed suggesting a sound basis for improved drug bioavailability and treatment of tuberculosis using the nebulized SLN formulation [97]. An interesting approach was adopted by Clemens et al. to target the TB-infected macrophages with functionalized mesoporous silica nanoparticles (MSNP). Two formulations were developed—a rifampicin-loaded MSNP coated with polyethyleneimine (PEI) and an isoniazid-loaded MSNP equipped with cyclodextrin-based pH-operated valve. In vitro experiments highlighted that the MSNP were internalized efficiently by the human macrophages and because of their functionalized nature, the MSNP escaped the acidic endosomes delivering the drugs into the cytoplasm. Thus, the functionalized MSNP demonstrated targeted intracellular delivery into the macrophages [93].

1.3.2.2 Malaria

Malaria is also a widely spread disease similar to TB with an estimated 216 million people affected in 2010 [98]. This disease is caused by the four species of the parasitic protozoans of the genus *Plasmodium*: *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*. Transmission of these parasites occurs through the bites of infected female *Anopheles* mosquito. The life cycle of the *Plasmodium* in the human host goes through the red blood cells (RBCs) and the hepatocytes making them the main parasitic targets. Moreover, the *P. falciparum* is involved in infecting the CNS often leading to fatality.

The current chemotherapy focuses on reducing the parasitic load in the infected cells and the choice of the drug is dependent on the *Plasmodium* species involved in infection. Even though chemotherapy is available, often complete treatment falls short. Apart from the factors related to the spread of the disease, drug resistance and the protozoan life cycle itself are responsible for the lack of complete therapy. Also, as with other drugs, side effects such as neuropsychiatric reactions, hypoglycemia, arrhythmia, hepatitis, agranulocytosis, anemia, and even life threatening reactions have been observed [14]. Because of the serious implications of the current therapy in terms of side effects and development of drug resistance, targeted drug delivery is important in the treatment of malaria. The knowledge that the parasites infect the RBCs and the hepatocytes mainly can be exploited by developing TDDS directed specifically towards them.

Chloroquine-resistant transporters develop as resistance mechanisms on the digestive vacuole membranes of the *Plasmodium*, the main site of action of chloroquine. A strategy to develop nanosystems sensitive to the pH difference in the intracellular compartments is suggested to avoid the chloroquine-resistant transporters and increase the drug payload in the infected cells. Chloroquine diphosphate was loaded into pH-sensitive liposomes and the release of the drug was estimated in vitro in simulated physiological pH conditions [14, 99]. Infected hepatocytes were actively targeted with liposomes incorporating peptides from the *Plasmodium* circumsporozoite protein in a series of experiments. It has been demonstrated that the liposomes accumulate rapidly and selectively in adult mouse livers. The targeting mechanism has been elucidated to be the binding of the targeted liposomes to the heparan sulfate proteoglycans in a fashion similar to the development of heparan sulfate immunoreactivity [100–102]. These proof-of-concept studies exhibit the possibility of development of a strategy to target the hepatocytes with antimalarials.

Halofantrine was intravenously injected in *P. berghei* infected mice as a formulation of nanocapsules prepared with either poly (D,L-lactide) (PLA) homopolymer or PEGylated PLA. The PEGylated nanocapsules were observed to be both long-circulating and cytotoxic to the parasites thus exhibiting passive targeting [103]. Considering that free halofantrine is usually involved in causing arrhythmia as a side effect, the nanocapsules would also benefit in avoiding such adverse reactions. In the case of *P. falciparum* infections, the CNS is usually infected. Accordingly, TDDS to overcome the BBB and facilitate passage of antimalarials would be beneficial to the therapy. The transferrin receptors in the BBB were targeted with transferrin-conjugated SLN loaded with quinine dihydrochloride. In vitro and

in vivo examinations showed higher percentage of the drug in the brain as compared to untargeted SLN loaded with the drug as well as free drug [104]. Other strategies that may be employed include antibody-directed targeted liposomes towards infected macrophages [105].

Recent vaccine development efforts have resulted in a new promising vaccine (RTS,S) for the first time for malaria (approval pending) [98, 106, 107]. This may lead to newer paradigms too in the prevention and treatment of malaria.

1.3.2.3 Human Immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS)

As per 2013 statistics provided by WHO, there are more than 35 million people worldwide who are infected with HIV/AIDS and the number keeps increasing. This disease is responsible for affecting the immune system the initial symptoms of which are like influenza and after a dormant period the immune system is severely compromised and in addition the patient is also exposed to opportunistic infections (e.g., TB, malaria) and even tumors.

The current antiretroviral therapy targets the steps along the life cycle of the HIV replication in the host cells. Briefly the steps are shown in Fig. 1.3 and include attachment and fusion of the HIV to host cell surface esp. $CD4^+$ cells, release of the viral core into the cell cytoplasm, reverse transcription of viral RNA into a double stranded DNA, integration into the host chromosome, protein synthesis and translation and followed by budding and release of mature virus into the extracellular region ready to

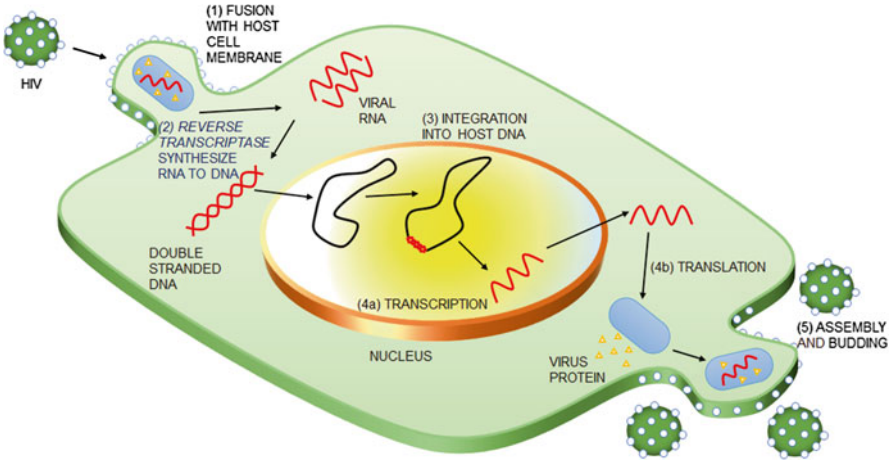

Fig. 1.3 HIV life cycle—(*1*) The first step involves fusion of the HIV with cell membrane of host cells expressing CD4+ and deliver the viral genome in the process. (*2*) Utilizing reverse transcriptase the viral RNA is reverse transcribed into a DNA which enters into the nucleus and (*3*) integrates with the host DNA. The next step leads towards viral protein synthesis (*4a*) and (*4b*) from where the viral proteins are assembled and after budding (*5*) off from the host mature HIV particles are released

affect other cells. The HIV-1 forms cellular reservoirs (dormant $CD4^+$ lymphocytes, macrophage, and dendritic cells) and anatomical reservoirs (CNS, male genitalia) [108, 109]. The current chemotherapy includes nucleoside reverse transcriptase inhibitors, nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and fusion and integrase inhibitors. Often, a combinatorial therapy is used referred to as highly active antiretroviral therapy (HAART). The use of such a chemotherapy has definitely aided patients in terms of improved survival rates but there are several deficiencies still haunting the HIV/AIDS patients. Frequent dosing, adverse reactions of the drugs, development of MDR, inaccessibility of the anatomical reservoirs are a few of the issues in these therapies.

On the other hand, the cycle of the HIV as well as its reservoirs in the body create an opportunity to develop TDDS against them. For example macrophage can be actively targeted with the help of targeting ligands attached to drug loaded nanocarriers against mannose receptor, formyl peptide receptor 1 and other similar receptors on the macrophages [110]. In such a manner the anatomical reservoir of HIV—the brain can also be targeted. Drug delivery to the brain is a difficult task and strategies for the same are explained in detail in later sections. As an example, water-soluble antiviral drug azidothymidine (AZT) was encapsulated within PEGylated nanoparticles with surface functionalization by transferrin. In vitro and in vivo evaluations confirmed that these TDDS targeted the transferrin receptors in the rat brains and enhanced the brain localization of AZT [111]. Another study was performed to actively target the lymphatic system where the HIV is known to colonize and form reservoirs. Liposomes loaded with zidovudine were surface modified with a lymphatic site-specific ligand—mannose—and compared against negatively and positively charged liposomes as well as unmodified liposomes. It was observed that the surface modified liposomes especially mannose coated were effective in uptake and localization into the lymph nodes and the spleen [112]. This study illustrated the benefits of targeting in improving the drug load in the lymphatic system to eradicate the HIV. Yet another study with poly(ethyleneoxide)-modified poly(epsilon-caprolactone) nanoparticles loaded with radiolabeled [^{3}H]-saquinavir demonstrated significant uptake and prolonged intracellular drug residence by macrophages in in vitro analysis [113]. Similar work of using TDDS in HIV/AIDS has been covered in these reviews [109, 114–116].

Thus, it is clearly observed that targeted drug delivery plays an important role in the therapy for infections.

1.4 Targeted Drug Delivery: Specific Location-Based Strategies

In this section challenges of targeted drug delivery to specific organs and organelles are discussed for which specific targeting strategies need to be employed as each of them presents specific challenges to drug delivery.

1.4.1 Blood–Brain Barrier (BBB) Targeted Delivery

The brain is a very difficult organ to deliver the drugs to because it is very well protected by the blood–brain barrier (BBB) and the blood–cerebrospinal fluid barrier (BCSFB). The BBB is highly specific in allowing transport and has two main functions by which it protects the brain and maintains its homeostasis: (1) supplying the brain with nutrients like glucose and (2) disallow harmful substances to pass through. As depicted in the Fig. 1.4, the BBB comprises an endothelial cell layer with tight intracellular junctions, a basement membrane and feet processes of pericytes and astrocytes. Apart from the tight endothelial junctions which form a physical barrier, the BBB also possesses enzymes and active energy-dependent efflux transporters which respectively inactivate the drug and exude the drug back into the blood from the endothelial cells [117]. The difficulty thus presented results in 98 % of small molecule drugs and almost 100 % of large-molecule drugs to not pass through the BBB to enter the brain. The only small molecule drugs that cross the BBB have high lipid solubility and a low molecular mass of <400–500 Da [118]. Still, the BBB consists of luminal and abluminal membranes which house the transport systems responsible for blood–brain and brain–blood transport of nutrients such as glucose, proteins, and peptides. In case of drug targeting such transport systems can be exploited to specifically target the drug to the brain.

As shown in the Fig. 1.5, different transport mechanisms responsible for transport across the BBB are [119]:

- paracellular transport (non-competitive movement of water soluble compounds through the tight epithelial junctions);
- transcellular transport (non-competitive movement of lipid soluble compounds through the epithelial cells from the luminal side to the abluminal side);

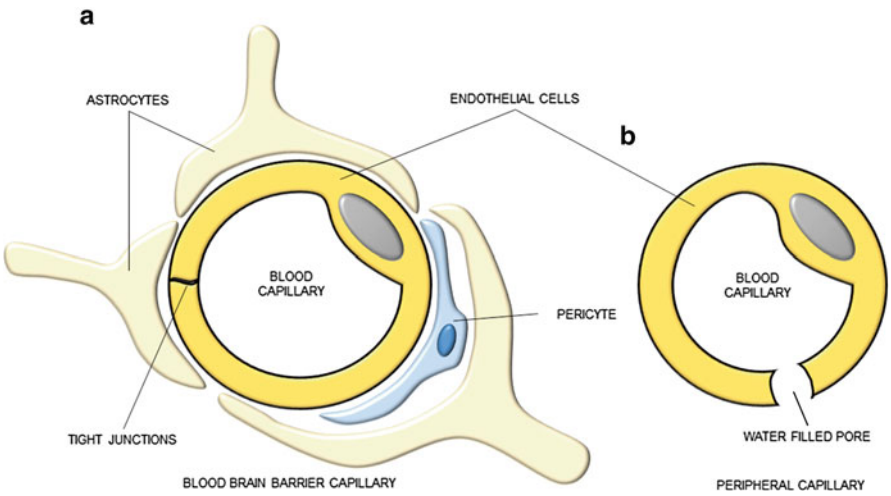

Fig. 1.4 Structure of blood–brain barrier (**a**) in comparison with peripheral capillaries (**b**)

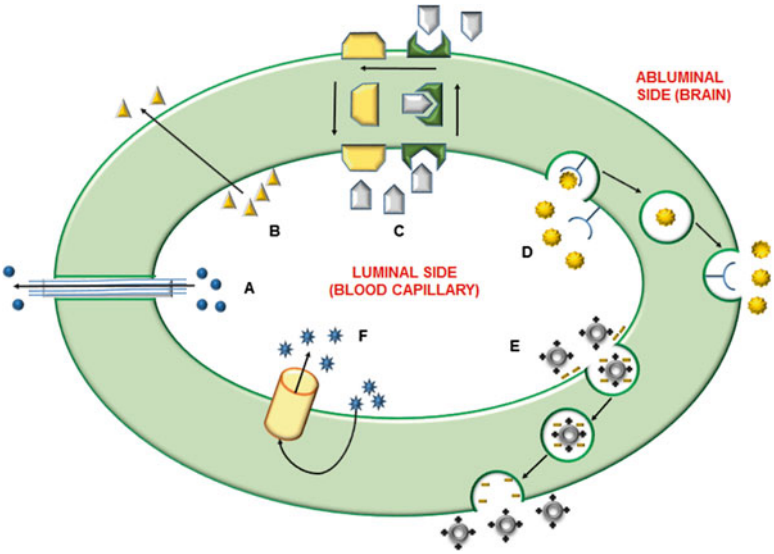

Fig. 1.5 BBB Transport—(**a**) Paracellular transport (water-soluble agents), (**b**) Transcellular pathway (lipid-soluble agents), (**c**) Carrier-mediated endocytosis (*CME* glucose, proteins, etc.), (**d**) Receptor-mediated endocytosis (*RME* transferrin, insulin), (**e**) Absorptive-mediated endocytosis (*AME* cationized agents) and (**f**) Active efflux transporter (drug substrates)

- carrier-mediated transport (CMT) (transport of compounds such as glucose, amino acids, proteins; also includes the efflux transporters on the luminal side like ABC transporters);
- receptor-mediated endocytosis (RME) (receptors for molecules like insulin and transferrin are present which transport the ligands on binding to the receptors);
- absorptive-mediated endocytosis (AME) (transport of plasma proteins like albumin after cationization).

Of course, diseased states like brain tumor, meningitis, infections among others can affect the structure and function of the BBB.

Drugs can be delivered to the brain by direct physical targeting and completely avoiding the BBB. The techniques employed for this include invasive strategies where the drugs are directly injected into the brain after drilling a hole in the head or implants carrying the therapeutics are surgically placed into the brain. Clinical studies were carried out with Gliadel® wafer containing carmustine (chemotherapeutic drug) implants in patients undergoing initial treatment for high-grade malignant glioma and it was observed that in combination with radiation therapy the Gliadel® wafers produced a survival advantage at 2 and 3 years confirming that the drug showed its effect best when it was delivered directly to the cerebral parenchyma [120]. Similar techniques have been used with devices such as Ommaya® reservoir pump, MiniMed PIMS® system, Medtronic SynchroMed® system, and DUROS™, among others [117, 121]. Utilizing the cerebrospinal fluid of the ventricles as a drug

depot via intraventricular injection is another invasive strategy employing the use of a catheter and relying on drug diffusion to various areas of the brain. Apart from delivering the drugs to the brain directly and avoiding the BBB, these methods also avoid the systemic exposure and related toxicity of the drugs as well as requirement of high doses to reach minimum therapeutic dose at the site of action. However, these systems come with their side effects, which include high chances of infections at the site of administration, catheter obstructions, cerebral edemas in case of high concentrations of the drugs in the parenchyma and massive patient discomfort in most cases. Moreover, there is the imminent cost of surgeries required for these delicate procedures and they would be required periodically when the implanted drug reservoirs get empty.

Non-physical targeting of the BBB and the brain which involves chemistry and biology based approaches, thus avoiding the surgical procedures, are also employed. One such technique relies on the temporary disruption of the BBB and increased passage of the concurrently administered therapeutic agents [117, 121, 122]. Administration of hypertonic/hyperosmotic solutions can cause shrinking of the endothelial cells and opening of the tight endothelial junctions due to osmotic pressure differences and the coadministered drugs can thus pass through. A commonly employed intracarotid delivery of a hypertonic solution of mannitol disrupts the BBB transiently and allows for the passage of the drugs [123–126]. BBB disruption has also been reported with the use of the mediators of the inflammatory response (leukotrienes, vasoactive peptides), bradykinin and alkylglycerols [127, 128]. In a way, such strategies are also invasive, although not physically, because they disrupt the natural BBB. They have showed potential in improved drug delivery but they also bring in the chances of exposure of the brain to the infectious agents and toxins and the possibilities of neuropathological changes like infarction, learning disabilities among others [122, 129].

Chemical modifications such as increasing lipophilicity of the drug can be exploited to deliver them via diffusion across the BBB. Of course, the drug still has to be of small molecular mass otherwise it will not cross the BBB. An example is the increased BBB delivery of highly lipid form of morphine: diacetylmorphine/heroin which is a prodrug form of morphine [130]. Nanocarriers such as solid lipid nanoparticles (SLN) or polymeric nanoparticles can also be used to improve the diffusion of the drugs across the BBB. Studies carried out by Yang et al. showed improved concentrations of camptothecin in the mouse brain when administered in the SLN as compared to the solution form of the drug. It was postulated that the improved concentrations were due to endocytosis and simple diffusion across the BBB [131].

The most promising BBB/brain targeted drug delivery is via the active targeting of the transporter mechanisms, namely the CMT, AME and the RME, and avoiding the efflux transporters on the BBB. The CMT in the BBB involves transporters/carriers such as GLUT1 (for glucose), LAT1 (for large neutral amino acids), CNT2 (for adenosine), MCT1 (for lactate) responsible for the BBB crossing of the respective nutrients [132]. Perhaps, the best example of CMT targeting is the use of L-DOPA, a prodrug of dopamine, in patients of Parkinson's disease which targets the LAT1 transporters and efficiently crosses the BBB [133]. After crossing the BBB,

the L-DOPA is converted to the active dopamine by decarboxylases in the abluminal side of the BBB effectively locking dopamine in the brain. Other examples include the use of melphalan (for brain cancer) and gabapentin (γ-amino acid) recognized by the LAT1 transporter.

A method of targeted delivery for large molecular drugs, such as proteins and peptides has been suggested. It involves cationization of the molecules or conjugation of the molecules with cationized albumin or cationized antibodies, thus forming chimeric peptides [134]. This process employs electrostatic interactions of the cationized drugs with the anionic charges on the luminal side of the BBB and the brain facilitating the AME transport of the drugs [135–137].

The BBB consists of receptors such as insulin receptor or transferrin receptor for the endogenous insulin, or transferrin, to transport the latter to the brain via RME. The expression of these receptors on the BBB is more than that in the normal cells, thus representing an opportunity to conjugate the therapeutic drugs or nanocarriers with targeting ligands or peptidomimetic antibodies and gain access to the BBB transport. Anti-transferrin receptor OX26 monoclonal antibody (mAB) has been the subject of several studies to conjugate the drugs as well as genes and target the transferrin receptor [138–141]. Humanized insulin receptor mAB was used by Pardridge et al. to demonstrate transport across the BBB in the Rhesus monkey and it can be used to target the insulin receptors [142, 143]. However, transferrin is present in high amounts endogenously which can compete with the targeted therapeutics while the insulin receptor targeting can also result in nonspecific effects in the body periphery [142]. Another well studied receptor for the RME across the BBB is the low density lipoprotein receptor (LDL). Surfactants such as polysorbate 80 have been attached to several nanocarriers to improve the BBB targeting and transport. When administered intravenously such surfactant-attached nanocarriers interact with plasma proteins like apolipoprotein E (APO E) which is recognized by the LDL receptors, and the targeted delivery is achieved [144–151]. Avidin/Biotin strategy as well as ADEPT has also been employed for targeted brain delivery [117, 152].

1.4.2 Targeting Drug Delivery to the Skin with Highlight on the Follicular Pathway

While the pharmaceutical market is flooded with thousands of formulations for skin delivery of drugs including free drugs in creams, ointments, lotions, dermal patches or sprays, this section focuses on targeted preparations for skin diseases. While the creams and similar preparations can be applied topically, the question needs to be asked whether the definition of targeted systems applies here. In case of free drug formulations, often the case is that the drug does not penetrate the skin because of the tight stratum corneum. Moreover, skin formulations like creams or lotions tend to wash away lowering the drug presence on the skin. Thus, formulations such as nanopreparations like liposomes, solid lipid nanoparticles, and dendrimers are studied to enhance permeation through skin and target the viable epidermis as well

as create stable drug reservoirs [153, 154]. Patients with acne, skin cancer, psoriasis, or infections can benefit from such preparations delivering drugs such as dithranol, miconazole nitrate, and isotretinoin, among others [155–161].

From past few years the *follicular pathway* has gained importance as a targeted drug delivery site and it is now considered as a subject of its own study apart from the topical/transdermal delivery. Initially, it was thought that the topically applied formulations would penetrate the stratum corneum, it has been seen that more penetration is observed through the hair follicles [154]. Of course, the follicular presentation occurs in a variable amount throughout the body (no hair follicles in the palms, soles of feet and the lips) with the highest follicular density observed in forehead and the sural making them one of the most accessible target sites. Targeting the follicular pathway demands an understanding of the pilosebaceous unit which is the integrated structure of the hair follicle, hair shaft, adjoining arrector pili muscle, and the associated sebaceous gland as shown in Fig. 1.6. The sebaceous glands as well as the bulge region are attractive target areas as the former is involved in diseases like acne, alopecia and anatomically capillary rich while the latter is rich in stem cells in charge of follicle reconstitution. Other targets can be the hair follicle infundibulum, the hair follicle papilla and the hair matrix [162].

It has been suggested that hair follicles in an active (open) state (sebum flow and/or hair growth) are accessible for penetration as compared to the inactive states. To improve the penetration, often pretreatments to remove the cellular debris from the stratum corneum are carried out with cyanoacrylate skin surface stripping (CSSS) [163–165]. Consideration must also be given to the phase of the hair growth cycle, i.e., anagen (growth phase), catagen (end of mitosis, cell death of lower follicle segment), telogen (resting phase), exogen (release of telogen fibers), and kenogen (time between exogen and subsequent anagen) while developing a delivery system [166, 167]. Furthermore, it was observed that systemic delivery through the follicular pathway was possible. Caffeine in shampoo applied topically to the skin

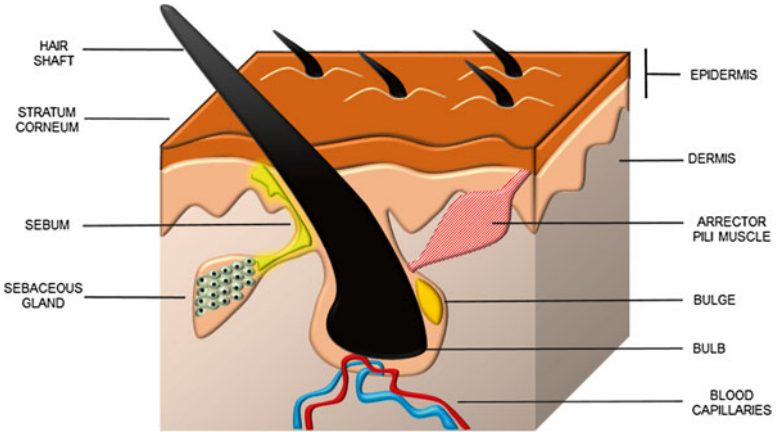

Fig. 1.6 Pilosebaceous unit

appeared in the blood 5 min later due to the presence of blood vessels around the sebaceous gland [168].

Studies exploiting the follicular pathway use liposomes generally because of the favorable characteristics, amphiphilic nature, and high loading capacities [169]. A recent study reported the use of minoxidil-loaded liposomes for pilosebaceous targeted delivery against alopecia areata. This study conducted the effect of charged liposomes on in vitro drug release, ex vivo skin permeation and drug retention behavior on rat skin. It was found that neutral liposomes showed maximum penetration and drug deposition in the pilosebaceous units compare to positively, negatively charged liposomes and non-liposomal formulation [170]. Monoclonal antibodies, DNA, vaccines in liposomes have also been studied for follicular pathway targeting [171–173].

Targeting the follicular pathway is still in its infancy and multiple safety studies should be carried out to prevent skin allergies or undesired systemic circulation of the drugs from the reservoirs in the sebaceous glands [174]. Still, the pilosebaceous unit is an important feature for topical delivery and should be studied further.

1.4.3 Pulmonary Targeted Drug Delivery

Respiratory diseases like asthma, tuberculosis, cystic fibrosis, lung cancer, and chronic obstructive pulmonary disease validate the use of delivering the drugs via lungs on top of which the anatomy and physiology of the respiratory system can be exploited for noninvasive, patient friendly systemic delivery of the drugs. The lungs provide a large surface area and a thin epithelial layer perfused with continuous blood flow. Targeting the lungs can provide quick systemic administration of the drugs and also assist in avoiding the first pass effect faced by oral drugs.

The pulmonary TDDS has to overcome barriers such as the mucus layer, alveolar lining fluid, epithelial cells, basement membrane, macrophages as well as enzymatic degradation. In the case of alveolar sacs the epithelial layer does not have tight junctions and as compared to the upper respiratory tract the rate of clearance is also less which makes them a good targeting location for drug/gene delivery [175, 176].

In treatment of diseases like asthma commonly used medications include anti-inflammatory drugs such as corticosteroids (beclomethasone, ciclesonide), beta-agonists (albuterol), anticholinergics delivered via inhalers and nebulizers. These long-term as well as immediate action systems are a preferred choice for several patients.

Current research in the pulmonary TDDS is highly concentrated on the use of nanocarriers especially liposomes and biodegradable polymeric nanoparticles. The important factors for efficient TDDS include size, shape and charge of the inhaled particles. For example, delivery to distal locations (lower respiratory tract) is favorable to particles with size around 1–5 μm, while larger particles will accumulate in the upper respiratory region. It has been noted that particles with size less than 1 μm are usually removed on exhalation [177]. Surface charge also plays a role

in how the particles interact after the inhalation as with low surface energy the particles will tend to aggregate less. The surface charge also determines the encapsulation efficiency of nanoparticles, their interaction with alveolar region and drug release [177]. Thus, optimization of physicochemical properties of the carriers forms a prime objective while developing the pulmonary TDDS.

Poly (DL-lactide-co-glycolide) (PLGA) microparticles containing rifampicin targeted against *Mycobacterium tuberculosis* infections were compared with "free" rifampicin in vivo in guinea pigs. The results not only highlighted that the aerosolized formulations reduced the infection but also that a single dose of the rifampicin in microparticles was comparable to daily doses of "free" rifampicin for 20 days [178]. In another study, treatment with aerosolized liposomal amphotericin B (AmBisome, Gilead Sciences, Foster City, California, USA) significantly improved survival compared to the aerosolized amphotericin B desoxycholate and placebo examined in immunocompromised rats with invasive pulmonary aspergillosis [179].

The group of Vyas [180, 181] carried out studies to develop aerosolized liposomes loaded with either rifampicin or amphotericin B against tuberculosis and aspergillosis infections respectively. The objective was to target the formulations to alveolar macrophages where the infection would be in densest form. The egg phosphatidylcholine (Egg PC) and cholesterol based liposomes were thus targeted to the alveolar macrophages by attaching macrophage-specific ligands (*O*-palmitoyl mannan, *O*-palmitoyl pullulan, *O*-steroyl amylopectin, and maleylated bovine serum albumin) or by imparting negative charges (with dicetylphosphate). Higher drug concentration in the lungs and preferential accumulation in the alveolar macrophages was observed in the targeted aerosolized formulations as compared to non-targeted liposomes as well as free drugs. Thus, Vyas highlighted the fact that ligand-attached liposomal aerosols had significant targeting potential.

Peptide and protein delivery is also studied as pulmonary TDDS with the benefit of large alveolar surface area and thin epithelium to aid absorption of the macromolecules. Perhaps, the most studied of such macromolecules is insulin. Multiple studies have been done with insulin-loaded microparticles to demonstrate efficient release in vitro and prolonged hypoglycemic effects in vivo in rats and guinea pigs [182–184]. It has also been shown that pulmonary TDDS can be utilized for gene delivery. A novel chitosan-based siRNA nanoparticle delivery system was developed by Howard et al. by complex formation between the siRNA and the chitosan polymers. The study of nasally delivered complexes demonstrated effective in vivo RNA interference in bronchiole epithelial cells of transgenic EGFP mice compared to the controls [185].

1.4.4 Retina

The drug delivery to the retina poses similar issues as brain drug delivery. This is because of the presence of the blood–retinal barrier (BRB), structurally similar to the BBB, which regulates the passage of the drugs to the retina from the blood.

The BRB too comprises tight junctions in its epithelium, a basement membrane as well as the presence of efflux transporter pumps such as P-glycoprotein (P-gp) [186, 187]. Thus, the delivery of drugs including small molecules, macromolecular peptides, and proteins is restricted into the retina. Targeted delivery to retina can comprise of physical targeting which are local (topical) and invasive methods and systemic targeting which is noninvasive where the therapeutic agents are administered systemically [188–190].

Topical administration involves the use of solutions and ointments as drug delivery systems and they are excellent choices for anterior segment of the eye and are patient friendly and cost appropriate too. But, these systems usually do not deliver drugs at effective levels at the retina which lies in the posterior parts of the eye [191, 192]. Along with that, the delivery systems are affected by drug loss due to washing off by tears, metabolism by the anterior segment enzymes and impermeability of the corneal epithelium [193]. Hence, as a route for targeted delivery to the retina, topical administration does not suit well.

Invasively administered drugs comprise intravitreal delivery, subconjunctival injections, and scleral implants. They avoid the barriers faced by the topical administration and are applied widely for targeted retinal delivery. Moreover, because the delivery is into the eye, systemic side effects are generally avoided. Definitely, the intravitreal injection is uncomfortable for the patient and frequent dosing is associated with high probabilities of injection associated infections and retinal detachment; still, advances are made to improve the dosing requirements and the drug presentation time at the retina by using nanocarriers and lipidic prodrugs [194–196]. Bourges et al. formulated polylactide (PLA) nanoparticles and observed targeted delivery and localization at the RPE cells after intravitreal injections in rats [197]. Another method is the use of intravitreal implants which can give sustained delivery for a longer period of time as compared to the injections; up to 6 months in case of implants compared to 2–3 times a week for injection. Such a delivery system is especially beneficial to patients with chronic eye disorders such as retinopathy. Vitrasert® is such an implant which is surgically inserted in the posterior region of the eye and delivers gancyclovir for up to 8 months. However, such inserts still carry the risk of loss of vision, vitreous haemorrhage, cataract formation and other adverse reactions [188]. Other invasive procedures employed include the scleral implants and subconjunctival injections which avoid the risk of retinal detachment associated with the procedures explained previously. Few studies have been carried out for scleral implants made from polymers such as poly (DL-lactide-co-glycolide) (PLGA) and ploy (DL-lactide) (PLA). Gancyclovir was delivered in these studies with sustained release of therapeutically effective doses obtained [198]. The subconjunctival injection beneath the conjunctiva enables the drugs to diffuse from there, through the sclera into the choroid [199].

In general the BRB is restrictive in allowing compounds to pass through except for nutrients. Hence, systemic administration results in very small amount of dose to reach the retina which is often below therapeutic levels. Higher dose administration results in systemic toxicity. Hyperosmotic mannitol injections can be employed to transiently disrupt the BRB and allow passage of the coadministered drug; but it

carries the risk of allowing infectious agents and toxins to pass through. Moreover the mannitol injections are responsible for BBB disruption too resulting in concurrent neurotoxicity. Now, the BRB has presence of transport systems similar to the BBB and they can be exploited for transport-mediated targeted drug delivery [190, 200]. Targeting ligands or antibodies can be attached to drug containing nanocarriers and prodrugs can also be made to achieve this purpose. Peptide transporters (PepTs) esp. PepT1 and PepT2 have been identified with broad substrate specificity towards dipeptides and tripeptides as well as peptidomimetics. PepT targeted 5′-amino acid ester prodrugs of nucleosides like gancyclovir, acyclovir, azidothymidine have driven increased bioavailability on oral administration [201]. Similarly, there has been observed presence of monocarboxylic acid transporters, folate transporters, and amino acid transporters on the BRB which can be utilized for targeted drug delivery [188, 190, 200].

1.4.5 Colon Targeted Drug Delivery

A number of diseases like inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis, colon cancer, irritable bowel syndrome (IBS), amoebiasis, etc. and desired transport of proteins and peptide drugs require the use of colon targeted drug delivery systems (CDDS). The general routes of reaching the colon are via the oral delivery or the rectal delivery. Using the rectal mode of administration is usually uncomfortable for the patient and can often result in irregular dose distribution. Conversely, using regular oral modes of delivery can degrade the drugs by acid actions in the stomach and alkaline and enzyme activity in the small intestine. Hence, for appropriate colon-specific delivery targeted systems should be utilized. So far, CDDS has seen the use of pH-dependent, time-dependent, and microflora-enzyme-dependent systems which have not proven to be foolproof. For example, it is possible that the pH-dependent system may survive the passage through the stomach but not the small intestine and the time-dependent system usually depends on the natural time for food and drug to passage through the gastrointestinal tract (GIT) which can be irregular esp. in the diseased states [202–204]. Improved technologies such as di-dependent systems utilize control by two factors to release the drug payload; for example, pH and time or pH and enzymes of microflora in the colon [205]. Ishibashi et al. developed three-layered capsule dosage form which consisted an acid-soluble polymer, a water-soluble polymer and an enteric polymer to deliver the active payload to the colon. Essentially this time- and pH-dependent dosage form was a predictable targeted system to deliver the drugs to the colon with high efficiency after in vitro and in vivo evaluations [206]. Yet another CDDS that depended on pH and microbes to deliver the drugs consisted of a traditional tablet core containing lactulose with additional layers of Eudragit E (acid soluble) and Eudragit L (enteric coat) on top of it, in that order, was developed to protect the active drug from the acid effects of the stomach (enteric coating), the alkaline pH of small intestine (acid soluble coating) and deliver to the colon wherein the lactulose would be

degraded by the colon bacteria. The enzymatic degradation of the lactulose would produce organic acids lowering the pH locally and dissolve the acid soluble coat releasing the drugs [207].

An alternative technique that can be used is to make prodrugs which provide protection in the upper GIT but undergoes enzymatic degradation in the colon to release the active drug. A study in the rats was performed using glycosidic prodrugs of dexamethasone and prednisolone. The prodrugs were not absorbed in the small intestine as they were hydrophilic thus reaching the colon intact. Once in the colon, the bacterial glycosidases cleaved the prodrugs to release the active drugs useful for targeting and treating the IBD in the colon. This study determined that the dexamethasone prodrug was better than the prednisolone prodrug [208, 209]. Moreover, it was suggested that modifications in the diet could induce the colon bacteria to produce specific enzymes which is a technique that can be used to further improve the efficiency of the delivery system. A large amount of interest has been seen in the development of azo-polymeric prodrugs to benefit from the azoreductase enzyme in the colon [210, 211]. Colazal® (Salix Pharmaceuticals Inc., North Carolina, USA) is an azo-prodrug of balsalazide indicated for ulcerative colitis.

The intrinsic ability of nanoparticles to accumulate at inflammation sites is also exploited for targeted delivery to the colon esp. in case of IBD. This results in long term deposition of the nanoparticles and drugs within at the site of inflammation [212].

1.4.6 Intracellular Targeting

In this section, the importance and strategies to carry out intracellular/subcellular targeting are highlighted. Once the therapeutics are able to reach the intended organ/tissue of action, they need to act either extracellularly or intracellularly. When the action is supposed to occur in extracellular regions, the task of arriving at the specific organ/tissue is enough. Yet when the mechanism of action of the therapeutic substance is on specific proteins, peptides, enzymes, nucleic acids (DNA/RNA) which are present within the cell, the TDDS needs to go a step or even two, in case of nuclear targeting, further to ensure that the specific drug/gene enter the cell and are in active form once they reach their intracellular targets. The targets in question may be located on the plasma membrane or cell components such as endosome, lysosome, endoplasmic reticulum, nucleus, mitochondria, or even mRNA binding complexes.

Plasma membrane targeting will be important for drugs whose actions are mediated through the proteins, lipids, signaling channels present on the plasma membrane. Targeting these drugs can facilitate high loading of the drugs around the cell increasing the effective concentrations where required. It also helps to reduce overall dose administered to the body. The plasma membrane has also been the subject of targeting in case of infections that depend on binding to the plasma membrane to initiate their life cycles. Fusion inhibitors class of HIV/AIDS drugs target and inhibit the HIV fusion and entry to the cells [213, 214].

The steps involved in intracellular targeting require the knowledge of how the cells can internalize components. Endocytosis is the process of absorbing molecules

by the cells, three ways of which are: phagocytosis, pinocytosis, and receptor-mediated endocytosis (RME). All of these processes lead into the endocytic pathway first step being the early endosomes responsible for sorting the internalized components and also mediating release of the receptors (in case of RME) for recycling. The early endosomes are characterized by mildly acidic pH. The early endosomes mature into the late endosomes or transfer the internalized components to the Golgi apparatus. Late endosomes also have mildly acidic pH (5.5) and result in formation of lysosomes after final sorting of the internalized material. Lysosomes are acidic and contain hydrolytic enzymes that degrade the material within. Definitely, the pathway consists of several signals and controls which are discussed in detail in this review [215]. Each of the steps in the endocytosis gives an opportunity to target.

Targeting the plasma membrane bound receptors (e.g., Tf receptor in tumor or brain) specifically allows binding to the intended cells of action which undergo RME and the TDDS are absorbed into the endosomes. They can be functionalized to endosomal markers to trigger the release of the drugs once inside the endosome. An acidic pH-sensitive system will, on entry into the endosomes, disintegrate to release the drug payload which can diffuse into the cytoplasm. Similarly, endosome-disrupting agents, which depend on the "proton sponge effect," can also be used to target the drugs/genes into the cells [216, 217]. The late endosome is responsible for trafficking the mannose-6-phosphate receptors and this can be a useful target for enzyme replacement therapy. Genetic disorders like Gaucher's and Fabry's require lysosomal enzyme replacement therapies where the mannose-6-phoshpate uptake can help target the enzyme replacements to the late endosomes and lysosomes [218]. Similarly, delivery of therapeutic substances to lysosomes was improved by lysosome-targeted nanosystems using lysosomotropic octadecyl-rhodamine B (RhB) [219, 220].

There are several drugs whose substrates lie in the cytoplasm. Even RNAi therapy requires that the siRNA be present in the cytoplasm to form the RNA-induced silencing complex (RISC). Hence, delivery of intact drug/gene to the cytoplasm is an important factor. As shown before, if the drug is internalized by endocytosis it is possible to initiate its release into the cytoplasm via endosome-disruption or stimulus responsive carriers. There is another technique which can deliver the drug/gene directly into the cytoplasm. It uses the cell penetrating peptides (CPPs) that transduce into the cells and directly release the payload into the cytosol. Several studies have been performed to explore this technology [221–224].

Multiple disease and disorders find their pathology to involve the role of mitochondria and its constituents. Consequently, drugs and nucleic acids with actions on mitochondria are useful bringing the question of targeting them to the mitochondria after entering the cytoplasm. A cytotoxic peptide (r7-kla) was made by conjugating mitochondrial membrane targeted fusion peptide (kla) with a cell-penetrating domain (r7) as an apoptosis inducer and an antitumor agent by causing targeted mitochondrial membrane disruption in both in vitro and in vivo experiments [225]. Other strategies may utilize similar targeting peptides for cell penetration and mitochondrial targeting to benefit patients of cancer as well as neurodegenerative diseases [226–232].

Gene therapy usually requires that the nucleic acids be delivered into the nucleus where the nuclear membrane forms an additional barrier. The usual approaches to gene therapy use viral-mediated as well as nonviral (e.g., liposomes) gene delivery which has been discussed in previous sections.

1.5 Summary

The field of medicine is filled with mires when it comes to safety and efficacy in applications of therapeutic substances to several diseases. Often the drug discovery provides potent leads but its research is not continued for the want of desirable physicochemical properties and absence of adverse effects. In case of some diseases such as cancer or HIV/AIDS it is not always possible to not use such drugs and while research of finding new class of drugs is ongoing, it is exciting to deliver the current drugs in a safe and efficacious fashion with targeted drug delivery systems. The advantage of such systems are to allow targeted deposition at intended site, sustained release, safety, reduced dosing frequency, and patient convenience. The goal of achieving efficient targeting has seen contributions from multiple fields like molecular biology, chemistry, and physics.

This chapter covered essential aspects of targeted drug delivery discussing challenges and strategies in several diseases and specific requirements of targeting at some locations in the body. Use of such strategies have led to improvement in disease conditions in several cases and underlines the importance of understanding the diseases, their physiology at tissue and molecular levels and identifying targets for developing TDDS.

While the TDDS has shown benefits in multiple conditions, further research is validated. The TDDS are not without pitfalls. For instance, while the targeting of cancer has shown several benefits in in vivo conditions, they are not able to completely cure cancer in humans. This is because cancer in humans is not just characterized by solid tumors, but metastatic cancer cells move around the body. Even if the solid tumors are targeted, the metastatic cancer cells may not be killed and cancer remission may be seen. Hence, targeting metastatic cells is also of prime importance. Other pitfalls attributed to targeting may include immune responses to antibody-directed therapies and the inability to achieve consistent pharmacokinetics when transferred from preclinical animal studies to clinical studies.

Still, the TDDS remain a viable approach to achieve efficacious treatments and continued exploration will lead to development of breakthrough therapies.

1.6 Conclusion

Thus, there exist both the variety of targets in the body and the variety of means to specifically bring pharmaceuticals to such targets. The past years have yielded significant preclinical data for several diseases. As we see throughout this chapter,

multiple studies have been carried out in vitro and in vivo to demonstrate the benefits of targeting drug delivery.

Major challenges exist in bringing TDDS from bench to bedside and continuous research needs to address them. One of the most important considerations is to understand the translation of preclinically proven TDDS into potential clinical material. Successful scale-up and industrial production of such systems, while keeping costs in check, will be the foremost step to see them in the clinics as well as individualized therapy.

The authors would like to highlight that this is an overview of different targeting strategies and would encourage readers to study each strategy in depth for better understanding.

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