Chapter 10
Pharmaceutical Nanotechnology: Overcoming Drug Delivery Challenges in Contemporary Medicine

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10.1 Challenges in Delivery of Contemporary Therapeutics

Drug discovery process has been in forefront utilizing recent advances in molecular biology, -together with medicinal chemistry, protein structure based screening, and computational analysis, as part of rational approach to discovering drug molecules that will address unmet clinical needs. For example, proteins identified from structural biology platform can serve as targets for discovering new drug molecules. The discovery of antisense oligonucleotides (ASN), plasmid DNA (pDNA), peptides and protein therapeutics has also shown a greater potential in treating several complex diseases. A recent development in drug discovery is RNA interference (RNAi) which uses small stretches of double stranded RNA with 21–23 nucleotides in length, to inhibit the expression of a gene of interest bearing its complementary sequence [1]. Small interfering RNA (siRNA) can induce RNAi in human cells. This RNAi technology has many advantages over other posttranscriptional gene silencing methods, such as gene knockouts and antisense technologies [2]. In addition, only a few molecules of siRNA need to enter a cell to inactivate a gene at almost any stage of development. MicroRNA (miRNA), advancement from siRNA, is a new class of drugs still in the investigative stage based on nucleic acid chemistry. miRNA with 19–25 nucleotides in length, interfere pathways that involve in disease process [3]. In general, all these recent drugs have shown a great potential in the clinical management of several complex diseases like cancer, metabolic diseases, auto-immune diseases, cardiovascular diseases, eye diseases, neurodegenerative disorders and other illness [1].

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Despite the diversity and size of therapeutic libraries are continually increasing, delivering them to the disease sites has been hampered by physico-chemical attributes of drugs and physiological barriers of the body. For example, many small and macromolecular drugs (ASN, pDNA, peptides, proteins, siRNA and miRNA) often fail to reach cellular targets because of several chemical and anatomical barriers that limit their entry into the cells [4, 5]. Therefore, the outcome of therapy with that contemporary therapeutics is often unpredictable, ranging from beneficial effects to lack of efficacy to serious adverse effects. These challenges have been discussed in the following sections with an attempt to apply nanotechnology-based concepts in designing of drug delivery systems (DDS) that overcome barriers in drug delivery.

10.1.1 Chemical Challenges

Physico-chemical properties impact on both pharmacokinetics and pharmacodynamics of the drug in vivo, and must be considered when selecting a suitable delivery method. The chemical challenges faced by small and macromolecular drugs (ASN, pDNA, peptides, proteins, siRNA and miRNA) are many folds, which mainly include:

(i) Molecular size
(ii) Charge
(iii) Hydrophobicity
(iv) In vivo stability
(v) Substrate to efflux transporters

Size, Charge and Hydrophobicity The chemical properties that mainly affect drug permeability through anatomical barriers are molecular size and solubility. High molecular size and increased hydrophobicity are the predominant problems particularly associated with combinatorial synthesis and high throughput screening methods [6]. These methods allow for identification of lead molecules faster based on their best fit into receptors, but shift the molecules towards high molecular size and increased hydrophobicity, resulting in poor aqueous solubility [6]. One estimate shows that around 40 % of the newly discovered molecules are poorly aqueous soluble, thus need a suitable delivery method to achieve pharmacologically relevant concentrations in the body [7, 8]. Oral route for drug delivery remains popular due to ease of administration and patient compliance. However, oral absorption can be hindered by poor aqueous solubility of therapeutics in GI fluids. The rate of dissolution, which is a prerequisite for oral absorption, depends on the drug solubility in the GI fluids. In addition, drug molecule must possess adequate lipophilicity (logP) in order to efficiently permeate across intestinal epithelial cells [9]. This is one of the reasons for hydrophilic macromolecules such as proteins, peptides, and nucleic acid constructs do not show any oral bioavailability and resulting in limited clinical success [10].

Drug transport mechanisms involving in intestinal epithelium are transcellular and paracellular transport [11]. Transcellular mechanism involving in transport of drug molecules across the cell membrane, which occurs by (1) passive diffusion, (2) facilitated diffusion, (3) active transport, and (4) transcytosis. Lipophilic drug molecules can diffuse freely across the epithelial membrane barrier while hydrophilic and charged molecules need specialized transport carriers to facilitate cellular uptake. Transcytosis process involving endocytosis and exocytosis mechanisms is mainly for macromolecular (proteins, peptides) drugs. Recent studies show that orally given nanoparticles can pass through the epithelial membranes in GI tract through the endocytosis process [12, 13], and this can be a potential route for transport of macromolecular therapeutics.

Paracellular route, on the other hand, involves diffusion of hydrophilic drugs between the cells of epithelial or endothelial membrane through sieving mechanism. The formation of tight junction between the epithelial and endothelial cells strictly limits the paracellular drug transport. Molecular cut-off for the paracellular transport is approximately 400–500 Da [14]. Molecular mass less than the cell junction can easily pass through paracellular route regardless of polarity, for example, water and ions. It has been observed that the diffusion of drugs with molecular size <300 Da is not significantly affected by the physicochemical properties of the drug, and which will mostly pass through aqueous channels of the membrane. However, the rate of permeation is highly dependent on molecular size for compounds with MW > 300 Da. The Lipinski rule of five suggest that an upper limit of 500 Da as being the limit for orally administered drugs [15]. Numerous studies are focused on identifying the nature of cellular tight junctions and the signaling molecules involved in preserving the barrier function in order to find right approach to promote oral drug absorption.

Increased hydrophobicity of a molecule also causes greater protein binding. Protein binding is both help and hindrance to the disposition of drugs in the body [16]. Elimination and metabolism may be delayed because of highly protein bind. Therefore, protein binding affects both the duration and intensity of drug action in the body.

Stability In vivo stability is also a critical chemical property of the drug that affects drug levels in the body. For example, the extent of drug ionization, stability in the acidic environment of the stomach or stability in the presence of gut enzymes, as well as presence of food and gastric emptying can reduce oral bioavailability of many small and macromolecular drugs. On the other hand, drugs are subjected to metabolism in the body by different sequential and competitive chemical mechanisms involving oxidation, reduction, hydrolysis (phase I reactions) and glucouronidation, sulfation, acetylation and methylation (phase II reactions). Cytochorme P450 enzymes which catalyze oxidation reaction are mainly responsible for first-pass biotransformation of majority of the drugs in the body, thus limiting oral absorption and systemic availability of the drugs [17]. Cytochrome P450 abundantly present in the intestinal epithelium and liver tissue, and metabolizes several chemically unrelated drugs from major therapeutic classes [17].

Besides this, macromolecular drugs such as proteins and peptides, ASN, pDNA, siRNA and miRNA have poor biological stability and a short half-life resulting in unpredictable pharmacokinetics and pharmacodynamics. Proteins and peptidal drugs are highly prone to enzymatic cleavage in the blood circulation and tissues, whereas nucleic acid therapeutics are highly susceptible to degradation by intra- and extra-cellular nucleases, leading to degradation and a short biological half-life [5, 18]. DDS have the potential to overcome the challenges of degradation and short biological half-life, and can provide safe and efficient delivery of macromolecular therapeutics.

Expression of Membrane-Bound Drug Efflux Pumps If the drug molecules are substrates to efflux pumps, their transport through cellular membranes is severely restricted [20–21]. The ATP-binding cassette (ABC) efflux pumps are transmembrane proteins present at various organ sites within the body, and use ATP as a source of energy to actively transport drug molecules across the lipid cell membranes [23]. Among the ABC family of efflux pumps, P-glycoprotein (P-gp) is highly expressed in epithelial cells of the small intestine, which is the primary site of absorption for the majority of the orally given drugs [24]. Efflux pumps also present on the luminal side of the endothelial cells of BBB, and restrict entry of hydrophobic molecules into the brain [25, 26]. The multi-drug resistance in many cancers is linked to the ABC efflux transporters which express on cell membranes and produce intracellular drug levels below the effective concentrations necessary for cytotoxicity [19]. All these efflux transporters preset a broad overlap in substrate specificities and act as a formidable barrier to drug absorption and availability at target sites [24].

DDS can be employed to overcome most of these chemical challenges. For example, paclitaxel is a potent anticancer drug, is poorly absorbed after oral administration and its bioavailability is <6 % [27]. The obvious reason for its low bioavailability are high molecular weight, poor aqueous solubility, the affinity to drug efflux pumps, and rapid metabolism by cytochrome P450 enzymes in the gut [24]. Nanoemulsions and self-emulsifying DDS have been employed recently for the successful oral and parenteral delivery of paclitaxel [20–22, 28, 29]. Similarly, to protect RNA based therapeutics from enzymatic cleavage, several DDS have been proposed and they are at different stages of preclinical and clinical development.

10.1.2 Remote Disease Targets

Anatomical and physiological barriers involved in the body restrict the direct entry of small and macromolecular drugs into the target extracellular or intracellular tissue locations [4, 30] resulting in sub-optimal doses at target site and reduced efficacy. However, cytotoxic drugs and RNA therapeutics have their target sites inside the cells, therefore need to be delivered intracellular in sufficient doses to produce therapeutic effect. The first limiting anatomical barrier for orally administered drugs is epithelial lining of gut walls, where from drugs will permeate through either by transcellular or paracellular transport. This transport is in turn dictated by the chemical properties of drugs as alluded above. Therefore, altering the chemical properties

by making the drugs in salt form, encapsulating in DDS based on cyclodextrins, lipid or polymeric carriers, or using permeability enhancers could promote bioavailability of drugs [20–22, 29]. Cytochrome P450 and efflux transporters present in the enterocytes of intestinal walls also forms as another limiting barrier to drug permeability [24]. Use of cytochrome P450 and efflux pump inhibitors can promote oral drug absorption. For example, pre-treatment with curcumin results in inhibition of P-gp and cytochrome P450 expression in the GI tract, leading to increased oral bioavailability and efficacy of drugs [20–22, 31].

For the drug molecules given intravenously, the limiting anatomical barrier is that of vascular endothelium and basement membrane. In addition, blood serum proteins, proteolytic enzymes, RNases etc. limit the effective drug delivery to the target sites [4, 30]. CNS disease are likely to rise to 14 % by 2020 mainly due to the ageing population, however, many newly discovered small and macromolecular therapeutics do not cross into the brain after systemic delivery [32]. Because brain is protected by blood-brain-barrier (BBB), which is composed of very tight endothelial cell junction and presence of several efflux transporters, resulting in formation of dynamic formidable barrier to drug transport [33]. However, once at the BBB, hydrophobic drugs with the size below <500 Da generally do transport through lipid cell membranes by passive diffusion, but if they are substrates to drug efflux pumps, they will be pumped out from the brain. Hydrophilic molecules also cannot transport efficiently as there is very limited paracellular transport present in the tight junctions of the BBB [20–22].

Cancer mass is another complex anatomical barrier in drug delivery. For example, solid tumor microenvironment is heterogeneous and structurally complex and presents a challenging barrier in drug delivery. The cytotoxic drugs which are intended to kill a large proportion of tumor cells in a solid tumor, must uniformly distribute through the vascular network, pass through capillary walls, and traverse the tumor tissue [34]. Nevertheless, the drug distribution in tumors is not uniform, and only a fraction of tumor cells is exposed to lethal doses of cytotoxic agents [34]. Tumor microenvironment is composed of tumor cells with varying proliferation rate and stromal cells (fibroblasts and inflammatory cells) that are surrounded in an extracellular matrix and nourished by a vascular network, and regions of hypoxia and acidity [34–37]. Each of these components may different from one site to another in the same tumor mass, and all of these factors effects tumor cell sensitivity to drug treatment [34]. In addition, stromal components in tumors contribute to an increase in interstitial fluid pressure, which limit the penetration of macromolecular drugs [38]. Furthermore, the three-dimensional nature of solid tumor tissue itself affects the sensitivity of constituent cells to chemo and radiation treatments [34, 39]. For instance, the tumor cells grown as spheroids in cell culture or tumors grown in animals, are more resistant to cisplatin and alkylating agents than the corresponding cell dispersions [40].

In addition, certain intracellular infections, like leishmaniasis and listeria, where macrophages are directly involved in the disease are not accessible to drug delivery, thus necessitating specific drug delivery strategies [41]. To overcome all these challenges, it is highly important to develop DDS that render protection to the drug from biodegradation in the body, while allowing their transport through the anatomic and physiological barriers to increase their bioavailability at the target tissue.

10.2 Nanotechnology Solutions

The science of nanotechnology has begun just in the last decade, but in this short time, it has been successfully applied in several fields ranging from electronics to engineering to medicine. Recent understanding of cellular barriers and molecular profile of diseases, and controlled manipulations of material at the nanometer length scale, nanotechnology offers great potential in the disease prevention, diagnosis, and treatment [30, 31]. Nanotechnology has also allowed for challenging innovations in drug delivery, which are in the process of transforming the delivery of drugs. Nanosystems fabricated using controlled manipulation of material are exploited for carrying the drug in a controlled manner from the site of administration to the target site in the body. They are colloidal carriers with dimensions <1,000 nm and can traverse through the small capillaries into a targeted organ down to target cell and intracellular compartments, which represent the most challenging barrier in drug targeting. The critical attributes of any nanoparticle DDS are to (1) protect a labile drug molecule from both in vitro and in vivo degradation, (2) maintain the effective pharmacokinetic and biodistribution pattern, (3) promote drug diffusion through the epithelium, and/or (4) enhance intracellular distribution. However, the specificity, sensitivity and simplicity are very important for any nanosystem to be clinically successful as a DDS. Several types of nanoparticle DDS have been evaluated for their potential drug delivery applications are in various stages of clinical development, these are discussed in the next sections.

10.2.1 Enhancing Solubility and Permeability

Solubility and permeability are two of the most critical biopharmaceutical characteristics impacting the successful delivery of drug molecules through anatomical membranes in the body. If the drug molecule is not a substrate to efflux transporters and metabolizing enzymes, then the solubility (hydrophilic and hydrophobic) plays a major role in determining oral intestinal permeability. Biopharmaceutical Classification System (BCS) is proposed based on the solubility and permeability properties of the drugs [42] which classifies drugs into one of four classes. Class I drugs are highly soluble and permeable in the GI tract, therefore, bioavailability is not an issue with Class I drugs. Class II drugs are poorly aqueous soluble but highly lipophilic. They are well permeable across the GI tract due to high lipophilicity, but the bioavailability is likely to dissolution rate limited due to low aqueous solubility. Class III drugs are highly soluble but have low permeability due to their low lipophilicity. In both Class II and Class III examples, DDS plays a critical role in overcoming poor solubility and permeability. On the other hand, Class IV drugs show low solubility and low permeability, and exhibit poor and variable bioavailability. Methods to enhance both solubility and permeability should be adopted for these drugs.

To improve solubility and permeability, several methods have been employed over the years. Such as preparation of prodrugs, use of chemical or physical permeability enhancers to transient openings of the tight junctions, or direct administration to the target site. However, formulation efforts can best exemplify in improving poor solubility and permeation profiles of both small and macromolecular drugs. Nanoparticle DDs like, liposomes, nanoemulsions, nanosuspensions, solid-lipid nanoparticles (SLN), micelles or polymeric nanoparticles are highly useful over the current methods to deliver the highly hydrophilic or highly lipophilic molecules across the intestines and BBB. For example, drug nanocrystal suspensions (nanosuspension) allow for increased dissolution velocity and saturation solubility of poorly aqueous soluble drugs, which is accompanied of an increase in oral bioavailability [43]. In addition, nanocrystals can be delivered intravenously for controlled drug release, and their surface can be tailored for both passive and active targeting. On the other hand, lipid-based systems like nanoemulsions and SLN could allow for the delivery of lipophilic drugs, by incorporating them in the lipid core of the formulation. These DDS can enable direct transfer of drug to the intestinal membranes and excluding the dissolution of drugs in aqueous fluids in GI tract. In once such study, we have formulated highly lipophilic paclitaxel into deoxycholic acid modified nanoemulsion, which showed increased oral bioavailability compared to paclitaxel solution [20–22, 44]. In another example, saquinavir, an anti-HIV protease inhibitor incorporated in nanoemulsion, showed enhanced oral absorption [45].

Recent studies show that nanoemulsions made using oils rich in omega-3 and omega-6 polyunsaturated fatty acids (PUFA) can promote drug delivery to the brain [45]. This is some extent attributed to the presence of PUFA transporters on the abluminal membrane side of the endothelial cells of BBB [46]. Tissue and cell permeability also altered by surface modification of the nanoparticles with targeting ligands which can facilitate the nanoparticle uptake along with its payload into the cells. These aspects have been discussed in the next sections.

10.2.2 Targeted Delivery to Disease Sites

Targeted delivery exploiting the structural changes and cellular markers of a given pathophysiology can potentially reduce the toxicity and increase the efficacy of drugs. This is highly important in case of diseases like cancer, where dose-limiting toxicities and drug resistance constitute major barriers to drug success. General targeting mechanisms consists of passive and active targeting [30].

Upon parenteral delivery, passive targeting depends on the size of the DDS and the disease vascular pathophysiology in order to preferentially accumulate the drug at the site of interest and avoid distribution to normal tissue [30]. For example, nanosystems escape from the blood circulation and accumulate in sites where the blood capillaries have open fenestrations as in the sinus endothelium of the liver [47] or when the integrity of the vascular endothelial membrane is perturbed by inflammation due to infections, rheumatoid arthritis or infarction [48] or by tumors [49]. In the liver, the size of capillary fenestrae can be as large as 150 nm [50] and

liposomal nanocarriers showed extravasation to hepatic parenchyma [47]. Nanosystems in the size range of 50–200 in size can extravasate and accumulate inside the tumor tissue and inflammatory sites [51, 52]. Therefore, the nanomedicine in the size range is expected to provide therapeutic benefits for treating these diseases. In case of solid tumors, passive targeting involves in the transport of nanosystems through a newly formed leaky tumor microvasculature into the tumor interstium and cells (Fig. 10.1). This phenomenon has named as "enhanced permeability and retention" (EPR) effect, first discovered in murine tumors for macromolecules accumulation by Maeda and Matsumura [53]. EPR effect is observed in many human solid tumors with the exception of hypovascular tumors (prostate or pancreatic cancer) [54, 55]. This effect will be optimal if nanosystems can escape reticulo-endothelial system (RES) and show longer circulation half-life in the blood. Poly(ethylene glycol) (PEG) grafting on nanosystems will evade RES uptake, allow for prolonged circulation in the blood and enhance tumor accumulation through EPR. Besides, the RES uptake of non-PEG grafted nanosystems also offers an opportunity for passive targeting against intracellular infections such as leishmaniasis, candiasis, and listeria which reside in macrophages [41].

The specificity of passive targeting can be remarkably improved when the targeting ligands are used with nanosystems, which selectively bind to cellular markers overexpressed on the disease site [56] termed as active targeting (Fig. 10.1). For example, folic acid-nanoparticles can be used to target tumor cells that over express folate receptors, such particles internalize via folate receptor mediated endocytosis [57]. In another example, arginine-glycine-aspartic acid (RGD) sequence containing peptides can be conjugated to nanoparticle to target $\alpha_5\beta_5$ or $\alpha_5\beta_3$ integrin receptors over express on endothelial cells of the newly formed angiogenic blood vessels and also on tumor cells. Furthermore, the targeting ligands anchored to nanosystems will allow for carrying of many drug molecules compared to direct conjugation of targeting ligands with drug molecules.

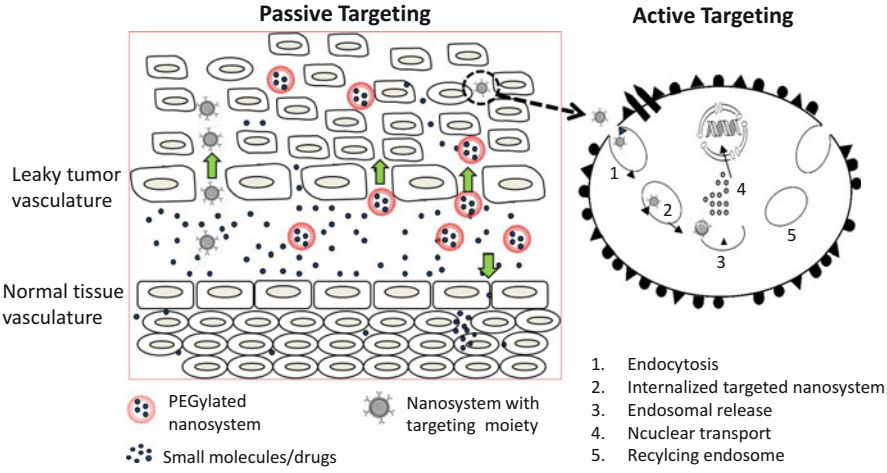

Fig. 10.1 Schematic illustration of passive and active targeting strategies in tumor drug delivery

10.2.3 Intracellular and Subcellular Delivery

The nanosystems once in the disease vicinity, they need to enter the cells and transfer the payload to sub-cellular organelles. There are two mechanisms playing a role in intracellular and subcellular delivery are non-specific or specific uptake of nanosystems by cells [30, 58]. In case of non-specific uptake, cells surround the nanosystems and forms a vesicle in the cell called an endosome. The endosomes then fuse with the highly acidic organelles called lysosome, which are rich in degrading enzymes. Endosomes usually travels in a specific direction and join at the nuclear membrane. Specific uptake on the other hand, involves receptor mediated endocytosis, where the actively targeted nanosystem binds to the cell-surface receptor, resulting in internalization of the entire nanoparticle-receptor complex and vesicular transport through the endosomes. The receptor can be re-cycled back to the cell surface following dissociation of complex. After the cellular internalization, stability of the payload in the cytosol and delivery to specific organelles, such as mitochondria, nucleus etc, is also essential for therapeutic activity. However, many drugs do not survive in the lysosomal environment. For example, 99 % of the internalized gene molecules undergoes degradation in endosomes. Thus buffering the endosomes for safe release of its contents helps in efficient gene delivery. Towards this, polycationic nanosystems have been explored, which causes endosomes to swell and burst, leading to the safe release of trapped content [59]. In another strategy, instead of trafficking drug carrier to the lysosome, the endosomal contents were released into the cytoplasm, thus bypassing the lysosomal degradation of the drug molecules [60, 61]. For example, a cyclic RGD functionalized polyplex micelles were taken into the cellular perinuclear space selectively through caveolae mediated endocytosis, thus escaping the lysosomal degradation of its active content [61].

Cellular uptake could be enhanced using of arginine rich cell penetrating peptides (CPP's) [62]. For example, HIV-1 Tat peptide was used to promote non-specific intracellular delivery of various therapeutics following systemic administration [63]. A number of cationic CPP's like penetratin also have been identified to promote intracellular drug delivery. In addition to intracellular delivery, use of delocalized cationic amphiphiles or mitochondriotropic nanosystems can promote mitochondrial drug delivery [64, 65].

10.2.4 Enabling Non-invasive Delivery

Non-invasive delivery is an alternate to systemic delivery of drugs, and mainly includes drug delivery via intranasal, pulmonary, transdermal, buccal/sublingual, oral and trans-ocular routes [66, 67]. Patient compliance has been found to be much higher when drugs given by non-invasive routes and therefore they are considered to be a preferred route of drug delivery. However, the preferred route of administration for a given drug selected based on several factors, such as biopharmaceutical properties (solubility, permeability and stability) of a drug molecule, disease state, onset of action, dose frequency and adverse effects. For example, sumatriptan and

zolmitriptan administered via intranasal route provide rapid-onset of relief from migraine related pain in minutes compared to oral tablet in hours. Similarly, potent peptidal drugs like calcitonin, desmopressin allows therapeutic blood levels that are not achieved with oral route of administration. In another example, selegiline and fentanyl transdermal products eliminate GI related adverse effects. In addition, non-invasive insulin products for inhalation and buccal administration improve patient compliance by reducing multiple daily injections.

In general, oral route is much convenient for high doses of administration. However, macromolecular drugs are not stable in the GI fluids, where intranasal, buccal/sublingual or pulmonary offers a non-invasive route of choice. These routes also favor treatments that need faster absorption of drug and where a rapid systemic exposure is well tolerated. Transdermal delivery is useful in chronically administered treatments (chronic pain, depression, Parkinson's, dementia, attention deficit-hyperactivity disorder and hormonal therapies), where sustained plasma profiles and low C_{max} to C_{min} ratio are required.

10.3 Illustrative Examples of Nanotechnology Products

Nanotechnology based concepts have been extensively applied in engineering of nanosystems for delivery of contemporary therapeutics in a controlled manner from the site of administration to the target disease in the body. The history of nanosystems reaches back to 1950s when the first polymer-drug conjugate was reported with N-vinyl pyrrolidine conjugated to glycyl-L-leucine-mescaline [68]. However, the most relevant nanosystems were conceptualized only after the first report of liposomal preparations in 1964 [69] and their subsequent use as vehicle for drug delivery application [70]. Soon after, synthesis of albumin nanoparticle was reported in early 1970s [71] with a subsequent early attempt of exploiting them as the first protein based DDS [72]. The pharmacological effects of polymer-based nanoparticles were studied [73] and their application as DDS envisioned around the same time [74]. As alluded earlier, ground breaking discovery of EPR effect in tumors by Matsumura and Maeda further emphasized on relevance of the size of delivery vehicle [53]. These seminal works drew tremendous attention on nanosystems for a sustained and controlled delivery of drugs. It was realized that for an optimized delivery system, the size of the payload vehicle should be between 10 and 100 nm. Kidneys easily clear off particles smaller than 10 nm while the particles larger than 100 nm are removed by the RES [73]. Since then, several different types of nanosystems have been researched and much focus has specifically been on tailoring the size, physical properties and surface functionality of the delivery systems for varying therapeutic applications. The collective research input on the nanotechnology based improvement of DDS has enabled several products in to the market in the past two decades (Table 10.1).

Sandimmune® and Taxol® are US Food and Drug Administration (FDA) approved dosage forms of cyclosporine and paclitaxel respectively, formulated

Table 10.1 Nanotechnology-based products in clinical application

Nanotechnology platform	Trade name	Active agent	Indication(s)	Approval year
Liposomes	Abelcet	Amphotericin B	Fungal infection	1995
	AmBisome	Amphotericin B	Fungal infection	1997
	Amphotec	Amphotericin B	Fungal infection	1996
	Daunoxome	Daunorubicin	Antineoplastic	1996
	DepoCyt	Cytarabin	Lymphomatous meningitis	1999
	Doxil/Caelyx	Doxorubicin	Antineoplastic	1995
	Myocet	Doxorubicin	Antineoplastic	2000
	OncoTCS	Vincristine	Non-Hodgkin's lymphoma	2004
Micelles	Estrasorb	Estradiol	Vasomotor symptoms	2003
Nanocrystal	Emend	Aprepitant	Antiemetic	2003
	Tricor	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia	2004
	Triglide	Fenofibrate	Hypercholesterolemia and hypertriglyceridemia	2005
	Megace ES	Magesterol acetate	Anorexia, cachexia or an unexplained significant weight loss in AIDS patients	2005
	Rapamune	Sirolimus	Immunosuppressant	2000
Nanoemulsion	Tocosol	Paclitaxel	Nonsuperficial urothelial cancer	2003
Nanoparticle	Abraxane	Paclitaxel	Metastatic breast cancer	2005
Nanotube	Somatuline depot	Lanreotide	Acromegaly	2007
Superparamagnetic iron oxide	Feraheme injection	Ferumoxytol	Treatment of iron deficiency anemia in patients with chronic kidney disease	2009
	Feridex	Ferumoxide	MRI contrast agent	1996
	GastroMARK	Ferumoxsil	Imaging of abdominal structures	1996

using Cremophor®EL as solubilizing nonionic surfactant. However, due to hypersensitivity reactions associated with these products, Cremophor®-free formulations based on nanosystems have been developed and commercialized. Genexol^{PM} is one such example of Cremophor-free polymeric micelles formulated paclitaxel where poly-(ethylene glycol) is used as a nonimmunogenic carrier while biodegradable poly-(D,L-Lactic acid) forms the drug solubilizing hydrophobic core [75, 76]. Several such DDS including liposomes, nanoemulsions, polymeric nanoparticles, micelles and nanocrystals (Fig. 10.2) have been developed, granted regulatory approval and have been marketed since then. The following section will focus on each of such DDS with illustrative examples of commercialized products.

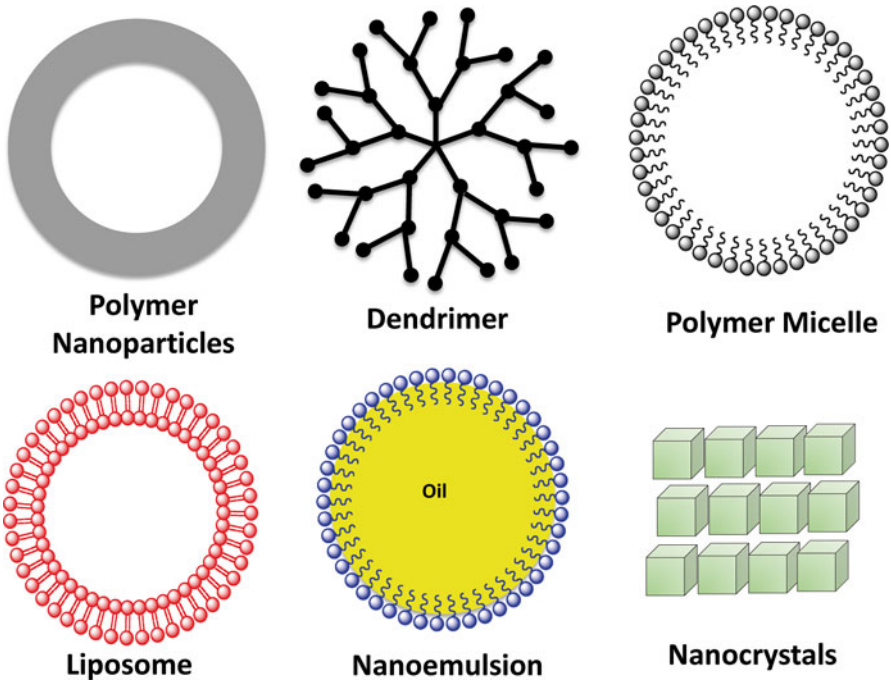

Fig. 10.2 Different types of pharmaceutical nanosystems used in drug and gene delivery

10.3.1 Lipid-Based Nanosystems

Lipid based carriers are extremely popular since they facilitate a controlled administration of both small and macromolecular drugs at therapeutically relevant doses. Liposomes and nanoemulsions are two most commonly used lipid based nanosystems for drug delivery application.

Liposomes Liposomes are vesicles formed of a lipid bi-layer, first developed by Alec Bangham in 1961, and their lipid bi-layer membrane is similar to that of cellular membranes. The lipid bi-layer of liposomes is composed of phospholipids with a hydrophilic head and a hydrophobic long-chain tail [77]. The hydrophilic core of the liposomes facilitates in compartmentalizing water-soluble drugs into the aqueous core while the hydrophobic bi-lipid membrane has been exploited to load water-insoluble drugs. Initial attempts using liposomes as nanosystems focused largely on improving their circulation time in the blood and targeting efficiency. PEG-modification of liposomes, first reported in 1990 [78] has by far been the most promising approach to achieve longer circulation of the liposomes in the blood. There has been a plethora of literature since then on the application of PEG-modified liposomes to achieve a selective delivery of drugs post-administration [79–81]. However, several other surface modifications of liposomes such as poly[N-(2-hydroxypropyl) methacrylamide] [81] poly-N-vinylpyrrolidones [82] polyvinyl

alcohol [83] and amino acid-based polymer–lipid conjugates [84] have been explored. Many studies showed that the opsonization of the liposomes might be dependent on the hydrophobicity of the surface, charge of the lipid and the molecular weight of the modifying polymer [85]. Antibody [86], folate [87] and peptide [88] mediated surface receptor targeting has been primarily enabled directing the liposome based drug delivery to the target organ.

The first liposome based formulation, PEG-liposome encapsulated doxorubicin was approved in 1995 (Doxil™, Orthobiotech) initially for the treatment of HIV-related Kaposi Sarcoma [89, 90] and later for ovarian cancer and myeloma. Doxil has remarkably reduced the cardiotoxicity by lowering cardiac exposure to free doxorubicin [77, 91]. Besides, it also increased half-life and tumor accumulation compared to free doxorubicin [92]. Furthermore, antibody modification of Doxil has shown a much higher tumor accumulation and enhances the cytoxicity of the doxorubicin [93]. In a study conducted on 53 patients suffering from advanced Kaposi's sarcoma, 19 patients showed partial and 1 patient showed complete response on administration of Doxil™ once every 3 weeks [94]. The success of liposomal doxorubicin has led to several liposomal-based drug formulations that are either approved for clinical application or are undergoing different phases of clinical trial. Some of the key drugs that have been exploited for liposomal formulation are shown in Table 10.1.

Nanoemulsions Nanoemulsions are heterogeneous system of two immiscible liquids; typically oil-in-water (o/w) or water-in-oil (w/o) with a droplet size in the range of 50–200 nm. These kinetically stabilized nano-sized droplets have several advantages over macroemulsions such as higher surface area and hence more free energy, higher stability with lower creaming effects, coalescence, flocculation and sedimentation [95]. The formation of nanoemulsions however requires an external shear force to decrease the droplet size to desired range and their productions methods are broadly classified as high-energy and low-energy methods. The high-energy methods could include laboratory or industrial scale high-pressure homogenization, microfluidization or laboratory scale ultrasonication [96]. However, these methods may not be conducive for applications involving thermolabile drugs, nucleic acids and proteins. Low-energy methods such as spontaneous emulsification, the solvent-diffusion method and the phase-inversion temperature (PIT) method are used for such payloads [95, 97]. The nanoemulsions serve as an excellent vehicle for solubilizing lipophilic drugs into the oil phase or hydrophilic drugs in the aqueous phase. The application of nanoemulsions as DDS has been envisaged only in the past decade and several attempts have been realized to increase their stability, circulation time and achieve a targeting efficiency [20–22, 98, 99]).

For example, propofol was first formulated in Cremophor® EL by Imperial Chemical Industries as ICI35868, and went into clinical use. However, due to the toxicity of Cremophor®, it was withdrawn from the market, reformulated in oil-in-water emulsion and launched by the trade name Diprivan® (ICI, now AstraZeneca). Apart from propofol as active pharmaceutical ingredient, the formulation contains generally regarded as safe grade excipients (GRAS) such as soyabean oil, glycerol, egg lecithin and disodium edetate [100]. Diprivan® is used as a short acting,

intravenous sedative used in intensive care medicine. It is known to have low toxicity, controlled sedation effect, rapid onset, a short duration of action and quick recovery despite prolonged usage [100, 101] TOCOSOL is another Cremophor® EL-free nanoemulsion formulation of paclitaxel that was approved by FDA in 2003 for the treatment of nonsuperficial urothelial cancer. Dexamethasone (Limethason®, Mitsubishi Pharmaceuticals), alprostadil palmitate (Liple®, Mitsubishi Pharmaceuticals), flurbiprofen axetil (Ropion®, Kaken Pharmaceuticals) and Vit A, D, E, K (Vitalipid®, Fresenius Kabi) are some other examples of therapeutically relevant compounds that have been formulated in nanoemulsions for clinical applications. Recently, NanoBio Corporation has formulated an emulsion-based antiviral drug NB 001 that shows potent activity against HSV-1 virus and antifungal drug NB 002 for the treatment of distal subungual onychomycosis (DSO). Both these formulations are currently in phase II/III trails.

10.3.2 Polymer-Based Nanosystems

Polymeric nanoparticles clearly are the most studied system for drug delivery applications. Different polymeric materials, natural, semi-synthetic and synthetic, have been exploited as polymer-drug conjugate or polymer-based nanoparticle for drug encapsulation to facilitate therapeutic applications. It is important to realize that while polymer-drug conjugate is a system which involves a single polymer chain conjugate to the drug, polymer-based nanoparticles are actually made up of several polymer chains which encapsulate the drug of interest.

Polymer-drug Conjugate Polymer-drug conjugates preparation date back to early 1950 [68] and the field has rapidly evolved since then [102]. Most drug molecules suffer from permeability through biological membranes, short half-life, non-specific distribution and dose dependent toxicities. Polymer conjugates on the contrary not only tremendously improves the in vivo circulation time of the drug but also facilitates passive delivery of these conjugates through leaky vasculature in diseases like cancer and inflammation [103]. They however also suffer from certain drawbacks such as polymer dependent toxicity, immunogenicity, rapid drug release, conjugate instability and poor drug loading. Several endeavors have been taken to overcome some of these shortcomings with much success. Besides, many bio-inspired polymers such as proteins (albumin, antibodies etc.) have also been looked upon as promising candidates for drug delivery applications.

The first polymer conjugate to undergo clinical trial was SMANCS where anti-tumor protein neocarzinostatin was (NCS) was covalently conjugated to two styrene maleic anhydride (SMA) [53]. SMANCS was approved subsequently in Japan in 1994 to treat advanced and recurrent hepatocellular carcinoma [104]. PEG-conjugate were the first candidate to get US FDA approval when PEG-L-asparaginase conjugate (Oncaspar) was accepted to treat acute lymphoblastic leukaemia [105]. Several other PEG -onjugates of drugs such as Neulasta (PEG-G-CSF; neutropaenia associated with cancer chemotherapy), PEG-asys (PEG-IFNα2a; Hepatitis B and C),

PEG-Intron (PEG-IFNα2b; Hepatitis C) have been approved to clinical treatment while several others are under various preclinical development. Besides, several other polymers (or their derivatives) conjugates (products names) such as polyglutamate (CT-2103, CT-2106), dextran (DOX-OXD, DE-310), N-(2-hydroxypropyl) methacrylamide (PK1, PK2, MAG-CPT, AP-5280, AP-4346) are being looked upon as promising candidates in their preclinical trial stages.

Though first protein nanoparticle based drug conjugation was reported in as early as 1974 [72] the first approved conjugate was realized only in 2005 when paclitaxel bound to albumin (Abraxane, AstraZeneca) was approved by FDA for treatment of metastatic breast cancer [106]. It is a non-targeted formulation with particle size around 130 nm, which is localized into the tumor partly through EPR effect and partly through albumin-binding protein. Clinical studies have demonstrated that Abraxane increases the therapeutic response, reduces the rate of disease progression and improves the survival rate among the patients. Antibodies have also been explored for drug conjugation and some examples of products from this class of nanovector includes Gemtuzumab (Mylotarg), Tositumomab and ibritumomab tiuxetan (Zevalin) [107, 108].

Micellar Delivery Systems Micelles are submicroscopic structures formed in an aqueous phase by amphiphilic surfactants or polymers that have a polar and a non-polar group. The typical size of these structures for delivery application ranges from 10 to 100 nm. These structures have a hydrophobic core, which facilitates the solubility of a lipophilic therapeutic agent and a hydrophilic corona that is exploited for surface functionalization to improve their tumor accumulation. These properties render them an attractive choice as carriers for drug delivery applications. Conventional surfactants however have a very high critical micellar concentration, and therefore are prone to disintegration on dilution in the blood stream [109]. Alternatively, polymeric micelles are usually prepared by self-assembly of a copolymer having hydrophobic moiety forming the biodegradable core while hydrophilic component for the surface. These polymers form micelles in aqueous media but at a much lower concentration compared to conventional surfactants [110]. Such polymeric micelles have been extensively researched for drug encapsulation, enhanced tumor targeting and longer in vivo circulation to aid an improved delivery system. Various approaches have been utilized to prepare polymeric micelles of desired properties using block copolymer, their lipid [111] or cyclodextrin [112] derivatives, diblock copolymers [113], triblock copolymers [114], pluronic polymer [115] and graft polymers [116].

Genexol-PM, a cremophor-free PLA-PEG copolymer-based micellar formulation completed its preclinical Phase I trial in 2004 [75]. Currently, the formulation is in its Phase II trial for the treatment of the patients suffering from taxane-pretreated recurrent breast cancer. SP1049C is another doxorubicin encapsulated pluronic polymer micelle based formulation that is under Phase II preclinical trial for the treatment of advanced level inoperable adenocarcinoma of esophagus [117]. NK911 is yet another example of a micellar formulation of PEG and doxorubicin conjugated poly (aspartic acid) which is under preclinical development [118].

Dendrimer Delivery System Dendrimers are roughly spherical nanoparticles made of several monomers, which branch out radially from the center. The advantages associated with dendrimers such as their controlled size, multiple valency, water solubility, modifiable functionality and an internal core render them a promising choice as drug carriers. They are therefore applied as delivery vehicles in several administration routes such as intra-venous, ocular, dermal and oral [119]. Their biocompatibility and immunogenicity has been studied in vitro as well as in vivo and similar to cationic macromolecules like liposomes and micelles, cationic surface groups render dendrimers cytotoxic to cells [120, 121]. Surface functionalization of dendrimers with PEG [122] or fetal calf serum [123] however has shown to reduce the cytotoxicity effects. The drug could be loaded on the dendrimers mainly by physical interaction or by covalent attachment. Physical adsorption of drug could suffer from poor drug loading and less control on drug release kinetics. Alternatively, the pro-drug approach is far more viable where the drug is chemically attached to the dendrimer directly or using a linker giving a much better pharmacokinetic and pharmacodynamic profile [124].

The field of dendrimer-based DDS has evolved greatly in the last decade and several dendrimer-drug conjugates are in their preclinical testing. One of the key examples is conjugation of PEO modified 2,2-bis (hydroxymethyl) propionic acid based biodegradable dendrimer to doxorubicin, which shows 9-fold higher tumor accumulation and 10-fold less cytotoxicity than free drug. The intra-venous administration of prodrug to doxorubicin-nonresponsive tumor showed a rapid tumor regression in a single dose [125]. Poly(glycerol-succinic acid) dendrimer (PGLSA)-camptothecin prodrug similarly has shown an enhanced solubility, cellular uptake and retention [126]. Since these initial success reports, several drugs such as artemether, cisplatin, diclofenac, mefenamic acid, dimethoxycurcumin, diflunisal, etoposide, ibuprofen, 5-florouracil, indomethacin and many more have been conjugated to dendrimer and are undergoing preclinical/clinical trials [127].

10.3.3 Nano-sized Drug Crystals

Poor aqueous solubility is one of the key problems with many small drug molecules, which affects their delivery and therapeutic applications. It is a well-established fact that with size reduction to nanometer scale, the properties of a material is governed by quantum laws and entirely different from its macro/micro size counterpart. A drug nanocrystal is therefore drug particle with its size in the nano-range i.e. 10–100 nm, and a suspension of such nanocrystals is popularly known as nanosuspension [219]. The suspension of these nanocrystals can be achieved in aqueous solutions as well as non-aqueous medium (liquid PEG, oil) with help of stabilizers like amphiphilic surfactants (poloxamers, PVP, phospholipids, polysorbate 80) or polymeric (hydroxypropyl methyl cellulose) materials. The hallmark of drug nanocrystals is that these crystals are pure drug particles with no carrier system. Similar to typical nanoparticle preparation, drug nanocrystals could be prepared by a

"bottom-up approach" (molecular level to nanocrystals) such as precipitation method or "top-down approach" (macro/micro level to nanocrystals) such as pearl milling (technology owned by Elan Nanosystems), high-pressure homogenization in water (technology owned by Skyepharma as well as Baxter) and in non-aqueous medium (technology owned by Pharmasol). Sometimes, a combination of the two approaches is used for nanocrystal production e.g. Nanoedge® (Baxter) that uses precipitation followed by homogenization. The major advantages of nanocrystallized drug are increased rate of drug dissolution and saturation solubility, improved oral bioavailability, reduced dose variations and general applicability to all routes of administration.

Rapamune® was the first nanocrystalline drug to obtain FDA approval in 2000 and was licensed to Wyeth Pharmaceuticals. It was produced by pearl milling method developed by Elan Nanosystems and contains rapamycin as the active drug. The formulation is marketed in two forms as tablets and oral suspensions. Soon after, Emend® was approved in 2003, which contains Aprepitant and is marketed by Merck. The production process has been developed by Elan Nanosystems and it is used for the treatment of emesis. Tricor® (drug Fenofibrate), Megace ES® (drug Megestrol acetate) and Theralux® (drug Thymectacin) are three other drugs which have been developed by Elan Nanosystems and have been licensed to Abbott, Par Pharmaceuticals and Celmed respectively. Several other products have however been introduced by other companies which include Semapimod® (Guanylhydrazone, Cytokine Pharmasciences), Paxceed® (Paclitaxel, Angiotech) and Nucryst® (Silver, Nucryst Pharmaceuticals).

10.4 Multifunctional Nanotechnology

As detailed in previous sections, biological system presents several barriers to effective drug delivery. It is therefore germane to develop drug delivery strategies to circumvent these barriers. This could be achieved by making the right choice of material as delivery vehicle, surface modification to increase targeting and intracellular availability of the drug and improving the functionality of the delivery system to achieve the diagnostic applications [128]. The nanosystem with these multifunctional abilities (Fig. 10.3) offer new possibilities in diagnosis, treatment and disease monitoring. The following sections will provide an in depth discussion on these aspects of drug delivery systems.

10.4.1 Choice of Materials for Nanotechnology

The material property of the delivery system is essentially the most important factor that governs the biocompatibility of formulation, stability and bioavailability of the drug and its clearance from the body. It is also equally important to

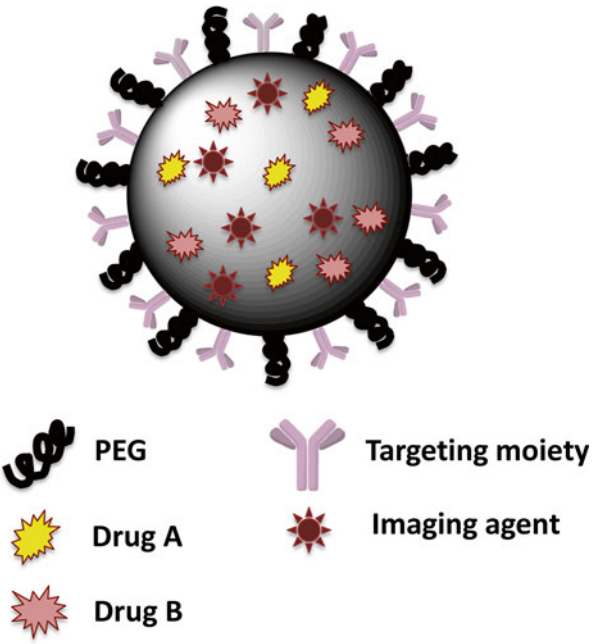

Fig. 10.3 A conceptual model of multifunctional nanomedicine with targeting ability, imaging capability, and drug/gene delivery in a single platform

understand the microenvironment of the target where the drug has to be delivered to achieve an effective therapeutic concentration. Design of nanosystems governed by microenvironment of the disease site results into a class of delivery systems that are popularly known as stimuli-responsive DDS. The delivery payload, route of administration and material safety profile, would also govern the components of such delivery vehicles.

pH Responsive Delivery Systems The physiological profile of an infected, cancerous or inflammatory body tissues differ drastically compared to the normal tissue. It has also been noted that various cellular compartments maintain their own characteristic pH levels; such as a lysosomal pH is around 4.5 where as in a mitochondria, the pH is around 8. These physiological differences result into a transmembrane pH gradient within the cellular compartments in a cell as well as among the cells. Such subtle differences in physiological environment could be actively exploited to design a pH responsive delivery system, which would be stable at physiological pH of 7.4 but actively degrade to release the drug under other conditions [129]. For example, a tumor is composed of rapidly dividing and metabolizing cells that are always short of the desired food and oxygen supply and thus rely on glycolytic pathways for harvesting energy to sustain [130]. The lack of oxygen in the tissue results in development of acidic condition within the tumor cells that could be exploited to achieve the delivery of a desired payload. The physicochemical properties of the delivery vehicles in response to difference in pH therefore are important characteristic, which has been actively focused in the past two decades.

Poly(β-amino ester) (PbAE) is a biodegradable cationic polymer which has been used for pH stimuli responsive delivery of drug. The polymer rapidly degrades under acidic environment with pH levels below 6.5 to release its payload into the cells. Significantly enhanced accumulation of drugs in the tumors has been demonstrated using PbAE polymer, leveraging pH stimuli-responsive delivery compared to a non-responsive polymer based delivery [131–133]. It has also been shown that the pH sensitivity of the polymeric delivery systems can be tailored by altering the length of the hydrophobic carbon chain length [134]. The pH responsiveness of poly (alkyl acrylic acid) polymer can be controlled by the choice of the monomer as well as the ratio of carboxylated to non-carboxylated alkylacrylate monomer. This polymeric system has been used for enhanced and effective in vitro transfection of lipoplex formulations. In yet another study, pullulan acetate-sulfadimethoxine polymer conjugate has been utilized to develop pH responsive, self-assembled hydrogels for an enhanced delivery of doxorubicin [135].

Polymers have also been directly conjugated to the target drug using pH responsive spacers, which would degrade under the low pH environment inside the tumors or lysosomes/endosomes to release the drug. In one such attempt, poly (vinylpyrrolidone-co-dimethyl maleicanhydride) (PVD) was conjugated to doxorubicin and its pH responsive controlled release increased the accumulation of the drug in to the tumor site [136]. Similarly, copolymer N-(2-hydroxypropyl) methacrylamide (HPMA) [137] and linear PEG based nanosystems are other candidates which have shown promise in delivery of drug to the tumor targets [138, 139]. Hydrolytically labile hydrazone linkage has been used for the drug release by enzymatic action in the lysosomes/endosomes from the polymeric or protein-based conjugate [138]. Serum albumin conjugates of anticancer drugs such as chlorambucil and anthracyclines have shown an enhanced antiproliferative activity compared to free drug [140]. Polyacetals are other pH labile candidates, which have been exploited for developing polymer based pH-responsive DDS [141].

Liposomes have similarly been suitably modified to achieve pH stimuli and controlled drug delivery. The intact pH-sensitive liposomes are internalized into the cells by endocytosis and fuse to the endosomes to deliver its contents inside the cytoplasm [77]. The desired modification of the liposomes is mainly achieved by using new lipid candidates, which provides acid sensitivity to liposomes or by conjugation of pH sensitive polymers on liposome surface to render them prone to pH sensitive degradation. Mildly acidic amphiphiles have been used to design such phosphotidylethanolamine based liposomes where at physiological pH, these amphiphiles act as stabilizers [142] but get protonated under acidic conditions causing a destabilization of the liposome and facilitating the delivery of the payload [143]. These delivery systems have been successfully researched to show in vitro delivery of antitumor drugs, toxins, DNA, antisense oligonucleotides and antigens [144]. Other lipids such as cholesteryl hemisuccinate (CHEMS), poly(organophosphazenes) and dioleoyl phosphatidyl ethanolamine (DOPE) have also been used for pH-sensitive liposomal formulations [145–147].

Micelles are yet another class of nanocarriers which have been extensively investigated to develop pH-responsive delivery. One approach to realize this aim has

been the employment of titratable amines or carboxylic groups on the copolymer surface such that the micelle formation relies on the protonation of these groups [148, 149]. In certain cases, water-soluble block copolymers exist in different forms depending on the pH of their aqueous solution and thus have been manipulated for drug delivery applications [150]. Besides, several other water-soluble copolymers have been extensively used to develop long circulating, pH responsive micelles. Some of the common examples include block copolymers based on poly[4- vinyl-benzoic acid (VBA) and 2-N-(morpholino)ethyl methacrylate (MEMA), poly (acrylic acid)-b-polystyrene-b-poly(4-vinyl pyridine) (PAA-b-PS-b-P4VP), Poly[2 (dimethylamino)ethylmethacrylate]-block-poly[2-(N-morpholino)ethyl methacrylate] (DEA-MEMA), poly(L-lactide)-b poly(2-ethyl-2-oxazoline)-b-poly(L-lactide) (PLLA-PEOz-PLLA) ABA triblock copolymers and diblock copolymers (PEOz-PLLA) etc., have been used for such applications [30].

Dendrimers are relatively new class of materials that are being investigated to develop pH-responsive delivery systems. One promising report has been the use of dendrimer composed of 2,2-bis(hydroxymethyl)propanoic acid monomer which has been conjugated to doxorubicin to produce a pH responsive delivery [151]. In another recent attempt, the terminal ends of core-forming PEO dendrimers have been modified with hydrophobic groups using acid-sensitive acetal groups. The hydrophobic groups are cleaved off the dendrimer in acidic environment resulting in the release of the drug [152].

Thermo-responsive Delivery Systems The cancerous cells are known to be highly fragile and sensitive to heat-induced damage (compared to normal cells) largely due to their rapid dividing nature. Incorporation of components that facilitate heat induction in presence of external stimuli such as magnetic field has therefore been looked upon as attractive choices to pursue. These facts have led to the development of hyperthermia as an adjunct to the radiation and chemotherapy for treatment of cancer cells. Several recent research efforts have shown that loading of superparamagnetic iron oxide particles to a delivery system leads to hyperthermia induced cell death at tumor sites [153, 154]. Use of drug delivery vehicle to localize these magnetic particles in the tumor sites ensure that only cancerous cells are subjected to elevated temperatures without affecting the normal cells. The tumor ablation by hyperthermia coupled with incorporation of an antitumor drug in the formulation leads to enhanced efficacy and accumulation of the drug [155, 156].

The thermo-sensitive polymers display a low critical solution temperature (LCST) in aqueous solution, below which they are water-soluble but become insoluble above it. This interesting property makes them an exciting choice as thermo-responsive DDS. One such example has been the accumulation of rhodamine– poly(N-isopropyl acrylamide-co-acrylamide) conjugate at the tumor site using targeted hyperthermia [156]. Certain amphiphilic polymers exhibit thermo-sensitivity where they have a temperature sensitive hydrophilic component and a hydrophobic component. Poly (N-isopropylacrylamide) (NIPAAm) and its other copolymers have been the most researched thermo-sensitive amphiphilic polymers [157]. In an interesting report, gold nanoparticles coated cross-linked Pluronic®

(poloxamer) micelles that showed a thermo-sensitive reversible swelling-shrinking behavior caused by hydrophobic interactions of copolymer chains in the micells [158]. Several other illustrations of such polymer based thermo-responsive nanocarriers have been accounted in details in literature for further reading [27].

Fabrication of temperature-sensitive liposomes has been an area of tremendous interest to the researchers due to the simple known fact that the membranes of different phospholipids are known to undergo phase-transition from gel-to-liquid crystalline and lamellar-to-hexagonal transition and are release small water-soluble components during such transitions. One popular example is use of dipalmitoylphosphatidylcholine as primary lipid for liposome formation. It shows a leaky behavior at gel-to-liquid transition at 41 °C and this transition can be tailored by adding distearoylphosphatidylcholine as a co-lipid [159]. Polymers have also been employed to design thermo-sensitive liposomes that also show LCST. These polymer chains exhibit a coil-to-globule transition with a change in temperature and thus impart temperature-regulated functionality to the liposomes [160]. Such polymers stabilize the liposomes in their hydrated form below the LCST but their dehydrated form destabilizes the liposomal structural integrity resulting in delivery of the drug [161]. Several reports exploit the modification of liposomes with NIPAAm copolymers for the fabrication of thermo-responsive substitutes [160, 162].

Redox-Responsive Delivery Systems Nucleic acid based therapeutics has acquired considerable interest lately and numerous attempts have been made to deliver ASN, pDNA, siRNA and miRNA, peptides and proteins for treatment of many genetic diseases. However, successful delivery of these biomolecules to the target cells is an important challenge considering the fact that these agents are highly prone to degradation. A stimuli-responsive system will be of tremendous application as DDS for these biomolecules to ascertain their structural integrity and therefore the therapeutic functionality. It has been established that there is a redox potential difference between the reducing extracellular space and the oxidizing intracellular compartment, which can be potentially exploited to guide the DDS into the cells [163]. Redox-sensitive delivery systems largely rely on components containing disulfide linkage that are taken up in the cell by endocytosis and the disulfide linkage is disrupted in the lysosomes to facilitate payload delivery [164]. The glutathione pathway plays a key role in reduction of the disulfide linkage in the reducing intracellular environment by maintaining an elevated level of reducing glutathione. Besides, the disulfide crosslinking also ensures more stable and robust structural integrity of the nanosystem that decreases the chances of early release of the payload.

One of the strategies to exploit the redox stimuli has been the use of polyaspartamide that uses positively charged groups in the polymer to electrostatically entrap DNA while the thiol groups on the polymer chain form the disulfide linkage resulting in formation of thiopolyplexes [165]. Thiolated gelatin particles have also been shown to form gelatin thiopolyplexes and have been used as potential redox-responsive nanosystem for pDNA delivery [166, 167]. Thiolated polyethylene imine has been directly conjugate to DNA to form polyplexes [168, 169] or have been used with a crosslinking agent [170] to successfully delivery DNA into the

cells with high transfection efficiency. In yet another report, glutathione sensitive polymer coated chitosan particles were used for designing of nanosystems stabilized by disulfide bond to provide gene delivery [171]. FDA has recently approved redox-responsive anti-DC33 antibody conjugate (Mylotarg®) for the treatment of acute myeloid leukemia [172].

Disulfide bond based redox-responsive liposomes have also been explored to enhance liposomal stability and delivery efficiency. Such liposomes are formed by a standard phospholipid along with a small chain lipid of which the hydrophobic and hydrophilic ends are linked by disulfide bond. These liposomes show tremendous structural stability until they reach the reducing environment inside the cells where the disulfide bond cleavage results in destabilization and delivery of the gene [173]. Thiocholesterol lipid based liposomes have been shown to successfully delivery gene into the cell in the reducing environment of the cells [174]. Mitomycin C conjugate with a cleavable disulfide bond incorporated into liposomes has shown lesser toxicity and better therapeutic potential than the free drug [175].

10.4.2 Surface Modification to Increase Availability at Tissue and Cell Levels

A careful designing of the nanosystems will enable them to deliver the drugs successfully to the target disease through active or passive targeting. However, to do so successfully, the DDS should be available in the blood stream for longer period of time by avoiding recognition by the components of immune system, circumventing the process of opsonization and preventing subsequent clearance by the RES. The longevity of nanosystem in the circulation not only allows their deposition at the target site through EPR effect but also improves targeting ligand to interact to its receptor. Suitable surface modifications of the nanocarriers for a prolonged and sustained presence in the body have therefore garnered tremendous interest.

Water-soluble polymers have been most commonly used to improve the retention time of the nanosystem in the blood and PEG is found to be most efficient in this regard. The PEG coating on the nanosystem surface provides a steric hindrance that prevents the interaction and binding of blood proteins to nanoparticle surface. The fact that RES recognition of a foreign object in the body largely depends on the binding on these plasma proteins to the surface, the sterically stabilized nanocarriers successfully escape body clearance [176]. This property to evade the immune system is popularly known as the "stealth" effect of the polymer. PEG is an excellent choice as surface protection moiety due to its high solubility in aqueous medium, flexibility of chain length, low immunogenicity and low toxicity. Besides, it does not interfere with the biological performance of the drug loaded in the delivery vehicle. PEG therefore by far is the most studied surface modifying agent to improve the residence time of the pharmaceutically relevant nanosystems. It has also been observed that while the particles modified with brush-like PEG effectively escape the immune response, surfaces modified with mushroom-like PEG molecules seem

to activate the immune system against the particles [177, 178]. Literature serves several derivatives of PEG that have actively been used to enable the surface functionalization of the delivery vehicles [179].

Besides PEG alone, copolymers of PEG have also been explored for surface modification of drug delivery constructs. Block copolymer of PEG-polylactide glycolide (PLGA) forms a hydrophobic core of PLGA and a hydrophilic shell of PEG that shows a longer residence time in the blood circulation [180]. Such polymeric preformed particles of PLGA could also be functionalized by PEG derivatives to prevent recognition by the immune system and therefore an enhanced retention time in the body. For example, the PLGA particles functionalized with polylysine-PEG copolymers shows a considerably reduced opsonization [181] while PEG modified poly (cyanoacrylate) particles provided longer-circulation as well as permeation into the brain tissue [182]. In a similar attempt, surface modification of polystyrene nanosystems by hydrophobically-modified dextran and PEG-dextran was studied to show that the stability of construct could be tailored by the density and also the nature of the surface modifying polymer [183]. Lipid derivatives of PEG have similarly been used to prepare PEG modified liposomes for enhanced circulation and improved performance of the delivery system [184].

Even though use of PEG has largely dominated the surface modification of DDS to increase retention time, several other alternatives have also been explored. The pre-requisite for a substitute of PEG has to be a water-soluble, biocompatible and non-immunogenic material. Polyoxomers, polyoxamines, polysorbate 80 and many more polymers have been used to modify the surface of nanoparticles to improve the bioavailability inside body. Lipid derivatives of poly (acryl amide) and poly (vinyl pyrrolidone) as well as other amphiphilic polymers such as poly (acryloyl morpholine) (PAcM), phospholipid (PE)-modified poly(2-methyl-2-oxazoline) or poly(2-ethyl-2-oxazoline), phosphatidyl poly glycerols, and polyvinyl alcohol have been successfully employed for surface modification of the liposomes.

10.4.3 Image-Guided Therapy

Imaging is an indispensable component of therapy and has been routinely used in hospitals and clinics for diagnosis of diseases and defects in the body. Conventional methods such as computerized axial tomography (CAT), magnetic resonance imaging (MRI), X-Ray imaging etc. have been employed in medical science for past several decades. Therefore, it was only fitting that with the advent of nanotechnology and more specifically nano-pharmaceutics, the concept of "molecular imaging" has been envisioned. Ability to image a DDS has therefore been an integral aspect of drug delivery application since it provides a visual feature to locate the site and extend of a disease in the body. Besides, it also enables a real-time assessment of the site of localization of a delivery vehicle in the body, its extent of sequestration in a particular organ and more specifically within a cell in question. For instance, presence of an imaging modality in a delivery vehicle customized to target a metastatic

tumor could be essentially tracked to the end site of its localization providing a direct visual evidence of the efficiency of a targeted or non-targeted system as well as the location of the tumor in the patient. Owing to the versatility of such a delivery system, extensive endeavors have been exercised to develop multifunctional nano-system (Fig. 10.3) comprising of targeting ligands, therapeutic agent(s) as well as imaging agents. To this date, several organic and inorganic imaging agents have been explored including liposomes [185], dye-conjugated silica [186], quantum dots [187], gold nanoparticle and nano-shells [188] magnetic nanoparticles [189] and many other contrast enhancing agents. Along with advances in conventional techniques like CAT and MRI scan, many new molecular imaging approaches such as radioactivity-based imaging (gamma scintigraphy, positron emission tomography (PET), single-photon emission computed tomography (SPECT)), surface enhanced raman scattering (SERS), optical coherence tomography (OCT), near-infrared fluorescence imaging etc., are been actively researched.

Radiolabelled probes are the most commonly used imaging agents in the drug delivery systems. Gamma scintigraphy provides a 2-dimension imaging ability while SPECT and PET enable a 3-D scanning. These techniques have their own advantages and disadvantages [190]. However, radioactivity based imaging systems are plagued by difficulties such as handling radioactive material, regulations concerned with their administration, their residence and clearance time from the body. Alternatively, improvement in MRI by the use of magnetic nanoparticles [191] or contrast enhancing agents [192] in the delivery system has been explored with vigor because of the non-invasive nature of the technique. Complexes of gadolinium, manganese, ferrofluids as well as superparamagnetic iron oxide are some of the most commonly applied contrast enhancing agents in MRI scans. Other popular imaging modalities include application of fluorescent dyes and quantum dots [193], SERS agents such as gold and silver nanoparticles [194].

10.4.4 Combination Therapeutics

Reports of multiples drug resistance (MDR) against antibacterial, antiviral, antifungal and anticancer drugs have become regularity in the previous decade. Numerous research endeavors have been applied to understand the origin of MDR and design therapeutic agents against them. However, the more we strive to overcome the medical enigmas by new drug discovery, the more complex the problem of MDR becomes. The gravity of the situation can be envisaged by a fact that the probability of MDR tuberculosis infection in acquired immunodeficiency syndrome (AIDS) patient is many folds more than a normal person. The inception of drug resistance has triggered the use of combination of drugs targeting a disease causing organism/process. The components of combination therapy may impact different independent targets, complement each other effect on the same target or bind independent of each other to give a combined effect for containment of the

disease. Such combination therapy has successfully been realized in the treatment of cancer, diabetes, bacterial and viral infections and asthma.

Co-administration of paclitaxel and ceramide using nanosystems has been proven to be extremely effective against MDR ovarian cancer [131, 132] as well as brain tumor cells [195] compared to the effect of individual drugs. Similarly, the use of a combination of paclitaxel and curcumin [28, 196] as well as doxorubicin and curcumin [197] enables to overcome the MDR in cancer cells. Several commercialized drugs such as Vytorin®, Caduet®, Lotrel®, Glucovance®, Avandamet®, Truvada®, Kaletra®, Rebetron®, Bactrim® and Advair® are actually a combination of two drugs [198]. Celetor Pharmaceuticals have developed CombiPlex® technology to launch combination chemotherapies for treatment of cancer. The technology uses high throughput screening, mathematical algorithm for synergy analysis and advanced nanosystems to predict right drug combination for therapy. This platform is meant to design chemotherapies so as to maintain an optimized ratio of the drugs in the body for enhanced efficacy. Their formulation CPX-1 is a fixed ratio combination of irinotecan and floxuridine that has shown positive results in its Phase-1 trial and is currently under Phase-2 trial for treatment against colorectal cancer [199]. CPX-351 similarly is a combination of cytarabine and daunorubicin and is under Phase-1 trial for the treatment of acute myeloid leukemia [200].

10.5 Regulatory Issues in Nano-pharmaceuticals

10.5.1 Approval of Pharmaceutical Products in the US

Despite the advances in nanomaterial application in disease diagnosis and drug delivery, significant amount of work still to be done in terms of characterizing nanomedicine safety and long term effects on biological system. Currently, all nanomedicine go through the FDA's traditional regulatory pathway within the Center for Drug Evaluation and Research (CDER) or Center for Devices and Radiological Health (CDRH). This pathway includes the following general requirements prior to approval.

(i) CDER reviews applications for new drugs.
(ii) Prior to clinical testing, laboratory and animal testing is performed to determine pharmacokinetic and pharmacodynamic attributes of the drug to determine a likely safety and toxicology profile in humans.
(iii) Clinical trials are performed in stages to determine if the drug is safe in healthy, then sick patients, and whether it provides a significant health benefit.
(iv) A team of FDA physicians, chemists, toxicologists, pharmacologists, and other pertinent scientists evaluates clinical data, and if safety and efficacy are established, the drug is approved for marketing.

Prior to the initiation of clinical trials, pre-clinical testing and manufacturing are regulated by several levels of regulation or guidance. These are FDA internally generated guidance documents, codified regulations listed in Title 21 Code of Federal Regulations (CFR) and International Conference on Harmonization (ICH) guidelines. Guidance documents are not codified law, but represent the Agency's current thinking on a particular subject. They do not create or confer any rights for, or on any person and do not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both [201].

Title 21 is the portion of the CFR that governs food and drugs within the United States for the FDA. It is divided into three chapters: Chapter I – Food and Drug Administration, Chapter II – Drug Enforcement Administration, and Chapter III – Office of National Drug Control Policy.

Most of the Chapter I regulations are based on the Federal Food, Drug, and Cosmetic Act. Notable sections in Chapter I are:

(a) 11 Electronic records and electronic signature related
(b) 50 Protection of human subjects in clinical trials
(c) 54 Financial Disclosure by Clinical Investigators [33]
(d) 56 Institutional Review Boards that oversee clinical trials
(e) 58 Good Laboratory Practices (GLP) for nonclinical studies

The 200 and 300 series sections are regulations pertaining to pharmaceuticals:

(a) 202–203 Drug advertising and marketing
(b) 210 cGMP's for pharmaceuticals
(c) 310 Requirements for new drugs
(d) 328 Specific requirements for over-the-counter (OTC) drugs

The 600 series covers biological products (e.g. vaccines, blood):

(a) 601 Licensing under section 351 of the Public Health Service Act
(b) 606 cGMP's for human blood and blood products

The 700 series includes the limited regulations on cosmetics:

(a) 701 Labeling requirements

The 800 series are for medical devices:

(a) 803 Medical Device Reporting
(b) 814 Premarket Approval of Medical Devices [104]
(c) 820 Quality system regulations (analogous to cGMP, but structured like ISO) [128]
(d) 860 Listing of specific approved devices and how they are classified

ICH guidelines are the result of The International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use and are unique in bringing together the regulatory authorities and pharmaceutical industry of Europe, Japan and the US to discuss scientific and technical aspects of drug

registration. Since its inception in 1990, ICH has evolved, through its ICH Global Cooperation Group, to respond to the global face of drug development, so that the benefits of international harmonization for better global health can be realized worldwide [202]. The FDA has adopted ICH guidance within four main categories as described below.

1. *ICH – Efficacy*
   (a) Clinical Safety E1–E2F
   (b) Clinical Study Reports E3
   (c) Dose-response Studies E4
   (d) Ethic factors E5
   (e) Good Clinical Practice E6
   (f) Clinical Trials E7–E11
   (g) Clinical Evaluation by therapeutic Category E12
   (h) Clinical Evaluation E14
   (i) Pharmacogenomics E15–E16
2. *ICH – Joint Safety/Efficacy (Multidisciplinary)*
   (a) MedDRA Terminology M1
   (b) Electronic Standards M2
   (c) Nonclinical Safety Studies M3
   (d) Common Technical Document M4
   (e) Data Elements and Standards for Drug Dictionaries M5
   (f) Gene Therapy M6
   (g) Genotoxic Impurities M7
   (h) Electronic Common Technical Document (eCTD) M8
3. *ICH – Quality*
   (a) Stability Q1A–Q1F
   (b) Analytical Validation Q2
   (c) Impurities Q3A–Q3D
   (d) Pharmacopoeias Q4–Q4B
   (e) Quality of Biotechnological Products Q5A–Q5E
   (f) Specifications Q6A–Q6B
   (g) Good Manufacturing Practice Q7
   (h) Pharmaceutical Development Q8
   (i) Quality Risk Management Q9
   (j) Pharmaceutical Quality System Q10
   (k) Development and Manufacture of Drug substance Q11
4. *ICH – Safety*
   (a) Carcinogenicity Studies S1A–S1C
   (b) Genotoxicity Studies S2
   (c) Toxicokinetics and Pharmacokinetics S3A–S3B
   (d) Toxicity Testing S4

(e) Reproductive Toxicology S5
(f) Biotechnology Products S6
(g) Pharmacology Studies S7A–S7B
(h) Immunotoxicology Studies S8
(i) Nonclinical Evaluation for Anticancer Pharmaceuticals S9
(j) Photo-safety Evaluations S10

The most relevant FDA regulatory document associate with nanomedicine manufacturing is the 'Liposome Drug Products' guidance document proposed in August of 2002 [203] This document currently guides development of liposomal based drugs, which generally fall into the definition of nanomedicine based on particle size. The guidance provides recommendations for drug development applicants on chemistry, manufacturing and controls (CMC), human pharmacokinetics and bioavailability; and labeling documentation for liposome drug products submitted in new drug applications (NDAs). The guidance recommendations are segmented as follows.

1. *Chemistry, Manufacturing, and Controls*

   (a) Description and composition
   (b) Physiochemical Properties
   (c) Description of Manufacturing Processes and Controls
   (d) Control of excipients: Lipid Components
   (e) Control of Drug Product Specifications
   (f) Stability
   (g) Changes in Manufacturing

2. *Human Pharmacokinetics and Bioavailability*

   (a) Bioanalytical Methods
   (b) In Vivo Integrity (Stability) Considerations
   (c) Protein Binding
   (d) In Vitro Stability
   (e) Pharmacokinetics and Bioavailability

3. *Labeling*

   (a) Product Name
   (b) Cautionary Notes and Warnings
   (c) Dosage Administration

Nanomedicine platforms have a number of common issues that are related to regulatory oversight. Some of these include functional qualities such as significantly different chemical properties than corresponding small or large molecules, different PK/PD/ADMET properties, delivery, targeting, release, stabilization, and bioavailability. Characterization, in terms of physiochemical attributes and general CMC issues (stability, sterility, etc.), are also common to many of the nanomedicine platforms, but differ greatly from the traditional small/large molecule drug [204].

While nanomedicine are becoming more prevalent in the areas of cancer, AIDS, and brain disorders, there are concerns that the unique properties of nanoparticles, such as size, shape, affinity, and surface chemistry may not fit the traditional safety and quality evaluation protocol proposed under current regulations.

The FDA and European Medicines Agency (EMA) have begun to address the lack of a more comprehensive regulatory framework for nanomedicine through the establishment of international scientific workshops such as the EMA 1st International Workshop on Nanomedicine in September of 2010 [205]. The FDA has also recognized the need for specific nanomedicine guidance, and is working toward that goal. In August 2006, the FDA established a Nanotechnology Task Force to determine the regulatory framework needed to develop safe and effective FDA-regulated products that use nanotechnology materials. The resulting Nanotechnology Task Force Report recommended that the FDA pursue the development of nanotechnology guidance for manufacturers and researchers, and that because of the emerging and uncertain nature of nanotechnology and the potential for multiple medical applications, there was a requirement for transparent, consistent and predictable regulatory pathways.

Current FDA recommendations, until specific guidance documents are developed, are to follow current FDA guidance including all normal testing procedures, normal drug stability testing, and those associated with CMC, in vivo, and in vitro analysis. Though understanding specific technical and scientific aspects of the drug product, tests should be designed accordingly. All parts of the drug product should be tested for stability, both individually and formulated. It will be critical for nanomedicine drug companies to communicate and develop acceptable procedures in concert with the FDA as early in the product development process as possible [204].

10.5.2 Preclinical and Clinical Development

There are more than twenty FDA approved products that contain nanomaterials (Table 10.1). To date, all of these products have been approved through the traditional regulatory pathway. As previously described nanomedicines are becoming more prevalent in the areas of cancer, AIDS, and brain disorders. There are currently hundreds of nanotechnology companies and research facilities trying to benefit from the emerging nanomedicine marketplace. Within the life sciences industry sector, funding has been primarily focused on those companies that apply nanotechnologies to ‘conventional’ therapeutics (i.e. drugs as either chemicals or biologics) to increase or extend their application; for example, targeted drug delivery systems (Nemucore Medical Innovations, BioDelivery Sciences International, CytImmune Sciences Inc., NanoBioMagnetics Inc., Nanobiotix, Nanotherapeutics Inc.), diagnostics (Nanosphere Inc., Oxonica Ltd) and medical imaging systems (Life Technologies Inc.- Qdots). These products and applications have a relatively

well-defined route to commercialization (subject to the regulatory hurdles facing nanotechnologies in general) [206].

Most notable of the nanomedicine products are the combinatorial drugs that combine targeting, drug delivery, stability, protection, and imaging. Figure 10.3 illustrates a typical combinatorial nanomedicine unit. The multifunctional nanoparticle is by nature a complex mixture of hydrophobic/hydrophilic molecules, inorganic components, peptides, and/or small molecule organic drug molecules. Many issues, regarding in vivo and in vitro assays need to be developed to segregate different properties of a multifunctional drug product. Some of these are:

(a) Synergies or interactions between the nanoparticle components
(b) Biocompatibility
(c) Long-term/chronic exposure assays/data
(d) General toxicology assays and analytics
(e) Animal models
(f) Molecular weight
(g) Particle size
(h) Charge distribution
(i) Purity
(j) Contaminants
(k) Stability – individual components and formulated
(l) Consistency in manufacturing
(m) PK/PD/ADMET assays/profiles
(n) Aseptic processing/sterilization
(o) Immunogenicity

10.5.3 Knowledge Management, Manufacturing and Scale-Up

Process development and manufacturing of nanomedicine is at its early stages of development and thus is also in its seminal stages of preparing to respond to the guidance of the FDA. With FDA's push to move from quality by testing to quality-by-design (QbD) (Fig. 10.4) for nanomedicine community to succeed in this new environment it is imperative to develop robust documented, process knowledge for the fabrication of nanomedicine. Acquisition and development of process knowledge will enable practitioners to bring novel therapies to the clinic with unique multifunctional capabilities. Articulation of the key variables (equipment, materials, idiosyncratic protocols etc.) at an early stage (i.e. the discovery lab) involved in production process will lead to a better understanding of how to translate good lab scale synthesis into scale processes for future clinical translation and assist manufacturing partners to produce material according to FDA's QbD principles.

QbD first implemented in pilot capacity by the FDA in 2005 has been formally adopted as a way to harmonize the development lifecycle of biopharmaceuticals and

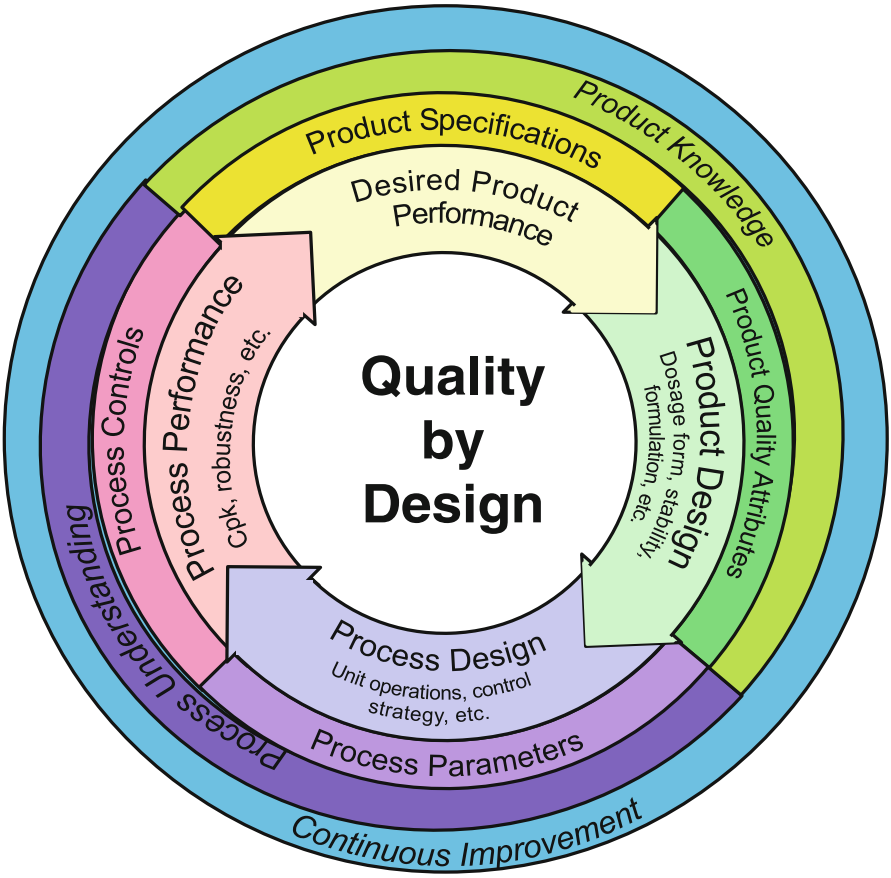

Fig. 10.4 The United States Food and Drug Administration recommended quality-by-design (QdB) approach to link product knowledge with process knowledge and create a continuous improvement product development environment

move away from sampling to find product defects to an environment were "in control" validated processes drive a data rich environment where variations within specification are acceptable. QbD is based on the underlying principle that quality, safety and efficacy must be designed into a product and that quality specifically cannot be tested or inspected into a product. Officially defined as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management" [207]. We consider QbD to be essential for the development of manufacturing processes for nanomedicine. QbD creates a continuous knowledge cycle, an important concept for advancing beyond the seminal steps for identifying innovative means to scale production of complex nanomedicine products [208]. The FDA

has come out with guidance that covers Pharmaceutical Development, Quality Risk Management, and Quality System with a predisposition that the future state of biopharmaceutical manufacturing, of which nanomedicine will be a part, will be an environment governed by QbD [207, 209]. Table 10.2 illustrates the differences in approach and the information requirements of QbD over traditional biopharmaceutical manufacturing which is dependent upon inspection, testing, locked processes and reproducibility [207].

It is important that nanomedicine manufacturers understand that QbD is knowledge rich environment dependent upon user definition of critical quality attributes (CQA), such that the physical, chemical, or biological property or characteristic of the intended nanomedicine should be within a proper range or distribution to ensure product quality. Linking CQA to process inputs (raw materials, chemicals, biologics etc), and process parameters (temperature, pressure, pH, etc) is performed in the early stage experimentation defined as the "design space" which is defined as the range of input variables or parameters for a single operation or it can span multiple operations. Early articulation of the design space, CQA and process inputs can provide a very flexible operational environment with the desired attributes for scale-up manufacturing.

Importance of Knowledge Management in Nanomedicine Nanomedicine holds the promise to cure complex diseases like cancer and save lives [213]. Today, academic scientists lead the development of the complex multifunctional nanomedicine, but for all their promise, there is a striking lag in clinical translation. This lag rests on the fact that nanomedicine investigators under appreciate the value of target product profiles (TPP), a key component of QbD, for ensuring that processes used in the laboratory are compatible with commercial scale-up processes and regulatory guidance [210]. A solution to this problem is at very early stage, put information into the hands of investigators to guide efforts towards nanomedicine that will have a chance to make it to the clinic. Innovation in informatics is another essential area and is complementary to the NIH's proposed investment to create National Center for Advancing Translational Sciences [211].

Nanomedicine translation faces substantial challenges related to managing the complex data streams emerging from the work at the bench, from process development work, and from preclinical studies all with important attributes required to drafting a TPP. The critical information developed during these activities is required to navigate a complex regulatory environment. Without effective data capture solutions and subsequent translation of large quantities of data into shared information, it will be "challenging" to coordinate the bench level process with scale-up process development, risk management and regulatory compliance. We are currently developing a software package, Fig. 10.5, designed to assist academics in overcoming this translational bottleneck for nanomedicine by consolidating existing drug development best practices into a single package for use as a guide to further advance nanomedicine development.

"Nanolytics", developed by Nemucore Medical Innovations, Inc. (NMI) is a knowledge management system for information pertinent to development of TPP, processes development plans, validation plans and risk management assessment

Table 10.2 Quality-by-design (QdB) approach in manufacturing

Aspect	Traditional approach	Enhanced QbD approach	Informatics requirements
Overall pharmaceutical development	a. Mainly empirical	a. Systematic, relating mechanistic understanding of input material attributes and process parameters to drug product CQAs	a. Knowledge management across entire life cycle
	b. Developmental research often conducted one variable at a time	b. Multivariate experiments to understand product and process	b. Process traceability and change management from development through manufacturing
		c. Establishment of design space	c. One-point access to all phases and all levels of data
		d. Process Analytical Technology (PAT) tools utilized	
Manufacturing process	a. Fixed	a. Adjustable within design space	a. Full documentation of process analysis and verification decisions
	b. Validation primarily based on initial full-scale batches	b. Lifecycle approach to validation and, ideally, continuous process verification	b. Integration of these decisions with PAT tool configuration setups
	c. Focus on optimization and reproducibility	c. Focus on control strategy and robustness	
		d. Use of statistical process control methods	
Process controls	a. In-process tests primarily for go/no go decisions	a. PAT tools utilized with appropriate feed forward and feedback controls	a. Web-based "Digital Dashboard" providing remote process monitoring
	b. Off-line analysis	b. Process operations tracked and trended to support continual improvement efforts post-approval	b. Record of all significant parameter variances and trends
Product specifications	a. Primary means of control	a. Part of the overall quality control strategy	a. All lifecycle documents (from URS though PBR) interlinked, with traceability of changes
	b. Based on batch data available at time of registration	b. Based on desired product performance with relevant supportive data	
Control strategy	a. Drug product quality controlled primarily by intermediate and end product testing	a. Drug product quality ensured by risk-based control strategy for well understood product and process	a. Inventories and risk assessments of all systems, equipment and processes
		b. Quality controls shifted upstream, with the possibility of real-time release or reduced end-product testing	b. Decisions on parameter prioritization and acceptable variances
			c. Integration with PAT records, for refinement analysis
Lifecycle management	a. Reactive (i.e., problem solving and corrective action)	a. Preventive action	a. Change management integrated across all lifecycle phases
		b. Continual improvement facilitated	

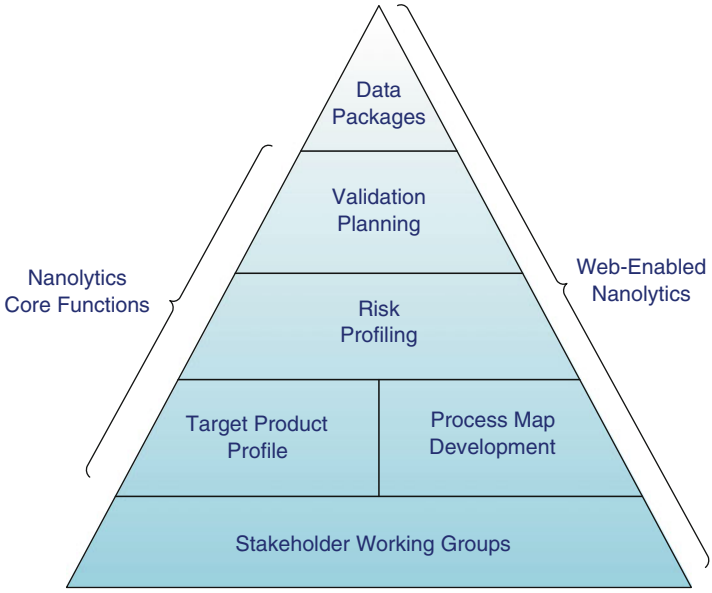

Fig. 10.5 "*Nanolytics*": Conceptual framework for combination of informatics with processing technology for optimization of nano-pharmaceutical formulations

needed to support effective nanomedicine translation. Nanolytics allows academic investigators early in research to contextualize how a nanomedicine could move to the clinic. Unlike either small molecule or biologic development the creation of nanomedicine, which are complex molecular entities, is very process and design intensive. A manual process already demonstrated value of an informatics approach to identify barriers (use of equipment not compatible with scale-up) and risks (regulatory, material, etc.) to translating these nanomedicine to the clinic. Nanolytics software consists of three suites: a TPP Suite, a Process Suite and Validation Suite. These suites and the knowledge they will manage should mitigate cost and reduce time of development of scale-up processes, lower barriers to clinical development for nanomedicine and leverage research costs more effectively [211, 212]. Nanolytics allows for the input of key information based on initial research and outputs documentation on how to achieve for the pilot scale production of the target nanomedicine. As always is the case, better information, begets a more realistic product development plans. This development of information "outside" of the typical areas of focus of a nanomedicine researcher will reduce risk and clarify efforts in translating nanomedicine from bench to bedside.

Significance to Nanomanufacturing Practices Developing manufacturing capability in the past has been capital intensive and typically relegated to a commercial

responsibility. But with many of the advances happening in nanomedicine there is a discreet need to lower the barrier to access manufacturing capabilities on a molecule agnostic platform. In an effort to create such an environment we have begun the process to establish the first in the nation FlexFactory™ nanomedicine manufacturing facility compliant with QbD principles. FlexFactory™ was developed by Xcellerex, Inc, (Marlborough, MA) to transition from single molecule manufacturing footprint to a modular, single use backbone which is agnostic to molecule. FlexFactory™ provides the ideal manufacturing environment for nanomedicine as the controlled environmental units (CEMs) are able to maintain a single unit operation of a manufacturing process with the ability to grow with the progress of the molecule from preclinical thru commercial launch. The innovation of the FlexFactory™, briefly, is if a unit operation needs to change for the development of a new nanomedicine manufacturing process the modular CEMs can be opened a new unit operation installed, the new step and the new process validated allowing for the production of a nanomedicine that conforms to different CQA. While there are other modular platforms that can be used in a similar manner they often have to be pieced together. The FlexFactory™ system has withstood numerous FDA audits, inspections, and license applications for a variety of biologics. The sophistication required for biologic therapeutic manufacturing is suspected to be similar to the complexity required for multifunctional nanomedicines. This level of complexity and novelty of scaling nanomedicine production is why we have taken a two-step approach to aggregate knowledge using Nanolytics and the molecule agnostic manufacturing platform FlexFactory™, Fig. 10.6.

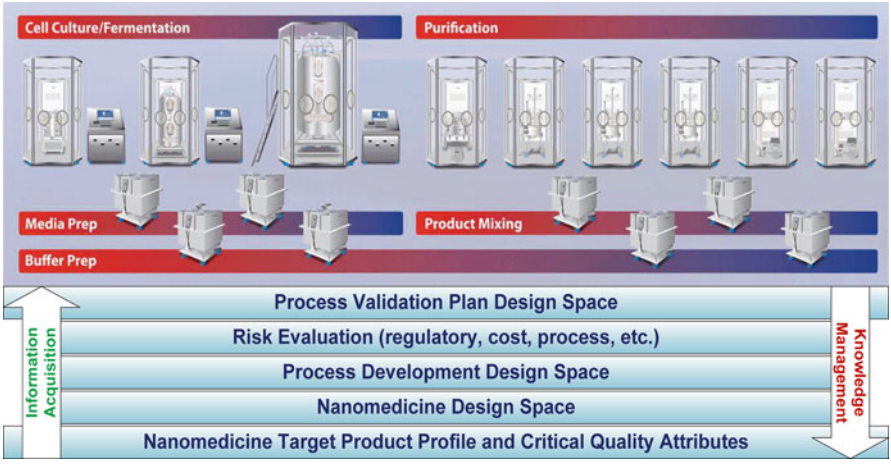

Fig. 10.6 NMI FlexFactory™ footprint shown to illustrate that data captured in Nanolytics serves as foundation for manufacturing Information and knowledge about product characteristics, process, and systems drive manufacturing design to optimize manufacturing of nanomedicine

10.6 Conclusions and Future Outlook

With greater understanding of chemical and physiological barriers associated in drug delivery and advances in nanomedicine design, there is an opportunity to efficient delivery of small and macromolecular drugs to complex diseases. Along these lines, the nanosystems have been engineered with specific attributes such as biocompatibility, suitable size and charge, longevity in blood circulation, targeting ability and image guided therapeutics, which can deliver the drug/imaging agent to the specific site of interest, based on active and passive targeting mechanisms. These systems cannot only improve the drug delivery to the target disease, but also the resolution of detection at cellular and sub-cellular levels.

To fully realize the potential of nanosystems for delivery of contemporary therapeutics in clinical setting, it is imperative that researchers also address the material safety, scale-up and quality control issues. Scale-up and quality control becomes extremely challenging especially when dealing with nanosystem designed to carry multiple drugs, imaging agents and targeting moieties. Furthermore, in vivo fate of nanomedicine engineered using novel nanomaterials are need to be fully assessed before being used in clinical application.

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