Chapter 10
Stimuli Responsive Carriers: Magnetically, Thermally and pH Assisted Drug Delivery

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Abbreviations

ABC	ATP-binding cassette
DEA	Diethylacrylamide
DOPE	Dioleoylphosphatidylethanolamine
ECM	Extracellular matrix
LCST	Lower critical solution temperature
NIPAM	*N*-isopropylacrylamide
PCLA	Poly(ε-caprolactone-co-lactide)
PAD	Poly(*N*-amidino)dodecyl acrylamide
PEO	Polyethylene oxide
PNIPAM–CS	Poly(*N*-isopropylacrylamide)–chitosan
NVCL	*N*-vinylcaprolactam
UCST	Upper critical solution temperature

10.1 Introduction

Clinical application of most of the drugs is limited by their side effects in spite of their beneficial action. There has been a long time desire to achieve selective delivery of bioactives to target areas in the body in order to maximize therapeutic potential and minimize side-effects. For achieving better therapeutic application,

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nanocarriers are considered for target-specific delivery of drugs to various sites in the body in order to improve the therapeutic efficacy, while minimizing undesirable side effects [1]. Nanocarriers possess in vivo longevity and specific capability of extravasation through the endothelium of inflammatory tissues (the so-called enhanced permeability and retention effect), whereas their functionalisation with biologically active ligands facilitates the targeting of specific cells. However, the translation of both the enhanced permeability and retention effect and ligand recognition into the clinic still remains questionable. This may be, to a certain extent a consequence of the stochastic nature of ligand–receptor interactions and of difficulties in the control of the release of the drug from targeting nanocarriers. One alternative involves on-demand processes (also termed "switch on/off"), which in principle allow for tailored release profiles with excellent spatial, temporal and dosage control. On-demand drug delivery is becoming feasible through the design of stimuli responsive systems that recognize their microenvironment and react in a dynamic way, mimicking the responsiveness of living organisms [2]. The concept of stimuli-responsive drug delivery was first suggested in the late 1970s with the use of thermosensitive liposomes for the local release of drugs through hyperthermia. Since then, and particularly in the past decade a great deal of research has been carried out on stimuli-responsive materials for drug delivery, especially concerning their design and applications as nanocarriers [3].

Stimuli-responsive nanocarriers are specialised nano-sized active delivery vehicles that evolve with an external signal and are equipped with "load-and-release" modalities within their constituting units. The central operating principle of these carriers lies in the fact that a specific cellular/extracellular stimulus of chemical, biochemical, or physical origin can modify the structural composition/conformation of the nanocarriers, thereby promoting release of the active species to specific biological environment. The observed changes are mainly decomposition, isomerisation, polymerisation, activation of supramolecular aggregation among many others. The general concept of triggered release can be divided mainly into two major modes according to the nature of the interaction between the bioactive molecule and the nanocarriers. In the complexation approach, where the bioactive agent is entrapped within the nanocarrier, the release can be triggered by structural change within the carrier scaffold (i.e. carrier degradation, cleavage of shell, charging of functional groups), while in the nanocarrier-conjugate approach; the mechanism of release involves the splitting of the linker between the carrier and the bioactive agent. The external stimuli which bring about these changes are numerous and cross related. These advanced nanocarriers thus become an active participant in the therapeutic landscape, rather than an inert carrier molecule [4].

10.2 Classification

Stimuli that trigger drug release from the nanocarriers can be broadly classified with respect to the biological systems as either endogenous (physiological, pathological, and patho-chemical conditions) or exogenous (physical) (Fig. 10.1).

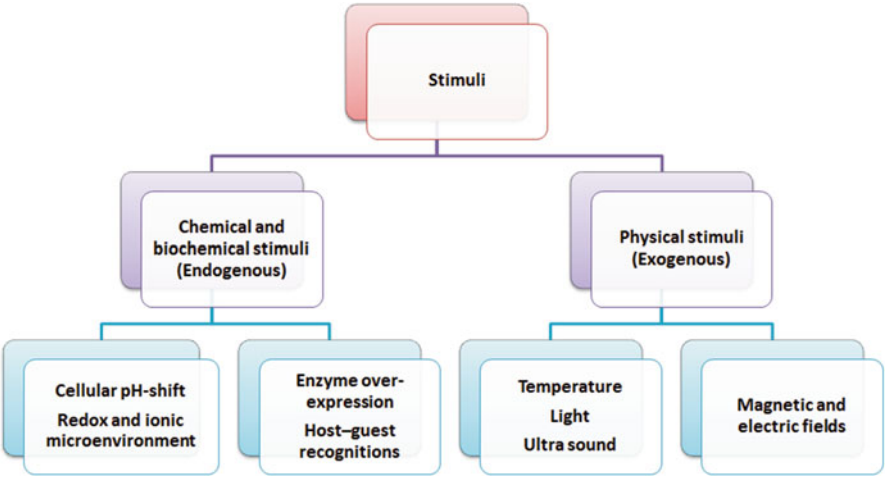

Fig. 10.1 Classification of stimuli involved in responsive drug delivery systems

Endogenous stimuli of chemical and biochemical origin include cellular pH-shift, redox, and ionic microenvironment of the specific tissues, enzyme over-expression in certain pathological states, host–guest recognitions, and antigen–antibody interactions. Physical stimuli that can be applied externally to bring about a triggered release of active guest may involve temperature, light, mechanical pressure, and strength of magnetic or electrical fields. This chapter throws light on pH, thermally and magnetic field assisted drug delivery either alone or as dual responsive systems.

10.3 pH-Responsive Carriers

Among the common stimuli, pH-responsiveness is the most frequently used. These carriers respond to pH gradients within the microenvironments of organs, tissues and cell organelles to achieve drug release at desired site. Certain tumours and inflamed tissue have a slightly lower pH values (between pH 5.4 and 7.4) than homeostatic conditions (pH 7.4). Furthermore, there exists a lower intracellular pH in endosomes and lysosomes. As such particles internalised through endocytosis will experience a pH gradient from neutral (pH ~ 7.4) in extracellular environment, to acidic (pH ~ 6.2) in early endosome and more acidic (pH ~ 5.0) in lysosome [5, 6]. Moreover, members of the ATP-binding cassette (ABC) efflux pump superfamily, such as P-glycoprotein (P-gp)/ABCB1, MDR-associated protein/ABCC, and breast cancer resistance protein/ABCG2, play important roles in drug kinetics including absorption, distribution, metabolism and excretion, which limits the accumulation of drugs inside the cells and results in drug resistance [7]. pH responsive carriers are expected to provide fast intracellular drug release and make the intracellular drug

concentration to reach a sufficiently high level to exceed the efflux capacity of drug transporters and the threshold concentration to kill the MDR tumour cells.

There are several general approaches of such systems that undergo chemical transitions around the critical pH range of 5–7 [8]. One approach is to introduce "titratable" or "protonizable" chemical groups such as amines and carboxylic acids into the components assembling the nanocarriers. The systems containing amines or carboxylic groups with different chemical structures and pKa values could change their physical and chemical properties, such as swelling ratio or solubility in response to local pH level [9]. Another approach is to incorporate acid-labile linkages directly to attach drugs covalently to the vectors or into the main-chains of the polymers constructing the carriers. The pH sensitive bonds are cleaved at acidic pH, accompanied by dissolution of carriers and release of drugs. The third approach is to incorporate carbon dioxide (CO_2)-generating ingredient for inducing CO_2 gas in acidic environment and leading to disintegration of the vehicles [10].

10.3.1 Delivery Systems

pH-responsive systems are mainly designed and reported as dendrimer, liposomes, nanoparticles and nanofibres (Fig. 10.2 and Table 10.1). Dendrimers are highly branched oligomers or polymers characterised by three structural features: (1) the central core from which the polymeric branches emanate, (2) the nature of the repeating unit which determines the microenvironment of the interior and thus the solubilisation ability of the dendrimer and (3) the nature and number of the terminal functional groups, mainly responsible for the behavior of dendrimers in solution. Pistolis et al. Developed pyrene loaded poly(propyleneimine) dendrimers for pH dependent release of pyrene. The release was increased up to tenfold by decreasing the pH to 2–4 [11]. Dual acting pH and thermosensitive dendrimer with a shell of poly(*N*,*N*-dimethylaminoethyl methacrylate) were also developed [12]. Similarly Yuan et al., reported pH-sensitiveness and cellular targeting dendrimer to provide the advantage of thermo regulated targeting. This system contained poly(L-glutamic acid) dendrimers with a polyhedral oligomeric silsesquioxane nanocubic core. Doxorubicin was attached via pH-sensitive hydrazine bonds and biotin was used as targeting moiety [13].

As another delivery system, pH-sensitive liposomes are designed to undergo acid-triggered destabilisation. For this, first generation pH-sensitive liposomes which are based on the cone-shaped lipid dioleoylphosphatidylethanolamine and later, serum-resistant pH-sensitive liposome formulations containing egg phosphatidylcholine and cholesteryl hemisuccinate are developed. These liposomes exhibited excellent stability at pH 7.4 and underwent rapid destabilisation upon acidification [14, 15].

For polymeric delivery, few common examples of pH sensitive polymers are poly(methacrylic acid)s, poly(vinylpyridine)s, poly(vinylimmidazole)s.

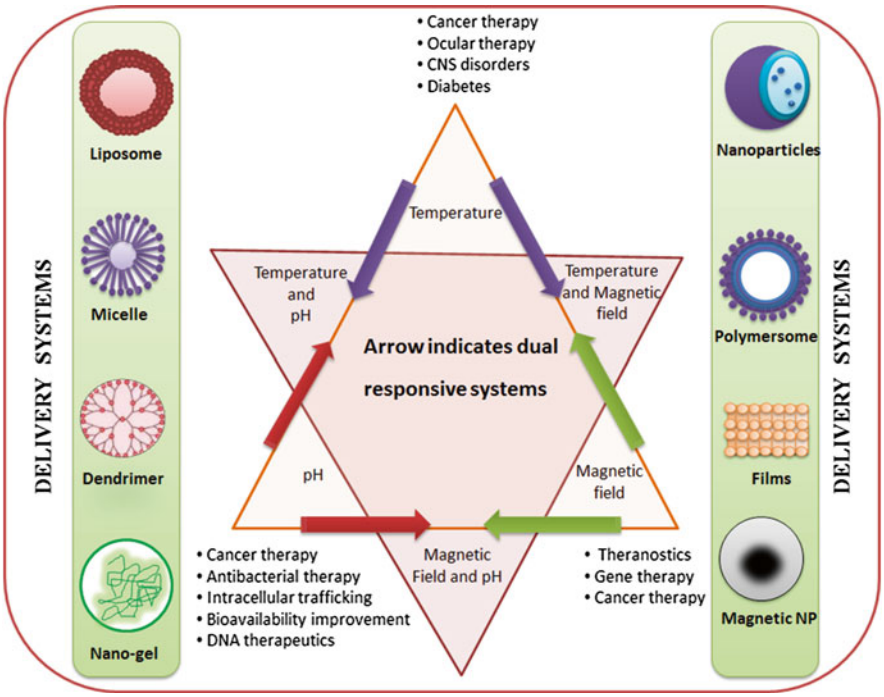

Fig. 10.2 Stimuli responsive carriers

Moreover, efforts are being made to develop new co-polymers with pH responsive properties. Giacomelli et al. Synthesised poly(ethyleneoxide)-b-poly(glycerolmonomethacrylate)–Indomethacin conjugates. This polymer–drug conjugate self assembled into micelle in water. The release of Indomethacin (IND) was governed by intrinsic molecular characteristics of free-IND (aqueous dissociation behavior) and pH-sensitivity of ester linkages in the conjugates. At neutral pH, the ester bond linkages were stable which promoted diffusion of free-IND out of the carrier, whereas acidic pH facilitates sustained release with slow kinetics [16].

pH sensitive poly(methacrylic acid and methacrylate) nanoparticles were designed to improve oral bioavailability of cyclosporin [17, 18]. Borchert et al. observed the pH-induced release of hydrophilic dyes from poly(2-vinylpyridine-b-ethylene oxide) block copolymer vesicles. At pH less than 5, protonation and dissolution of the poly-2-vinylpyridine blocks facilitated the release of dye from vesicles [19]. Similarly, gold-decorated shape-persistent, pH-responsive polymersomes were prepared by the self-assembly of a novel poly(ethyleneoxide)-block-poly[2-(diethylamino)ethylmethacrylate-stat-3-(trimethoxysilyl)-propylmethacrylate], copolymer. These pH-sensitive blocks are located in the membrane walls, while the hydrophilic PEO chains forms the corona [20].

Table 10.1 Polymers and systems used for different stimuli responsive carriers

S No.	Polymer	System	Outcome	Ref
pH responsive carriers				
1	Poly(L-glutamic acid)-b-poly(butadiene)	Polymersome	pH-triggered size change was observed	[106, 107]
2	Poly(ethylene glycol)-b-poly(glycerol monomethacrylate)-Indomethacin	Copolymer–drug conjugate	Acidic pH facilitated sustained release with slow kinetics	[16]
3	Poly(ethylene glycol)-b-poly(2-vinylpyridine)	Polymersome	Fluorescein/pH-triggered	[19]
4	poly(L-lysine)-b-poly(leucine)	Polymersome	Biodegradable pH responsive polymer	[108]
5	Poly(2-(methacryloyloxy) ethyl phosphorylcholine)-b-poly(2-(diisopropylamino) ethyl methacrylate)	Polymersome	DNA release at pH less than 6	[109]
6	Poly(ethylene glycol)-b-poly(styrene)-b-poly (2-diethylaminoethyl methacrylate)	Polymersome	pH-tunable membrane permeability	[100]
7	Poly(L-lysine)-b-poly(L-GLUTAMIC acid)	Micelles and vesicles	Biodegradable pH responsive polymer	[110]
8	Linear copolymers of ethylene and acrylic acid	Nanoparticles	Nanoparticles dispersed in aqueous media exhibited remarkable reversible thermoresponsive behaviour upon heating/cooling from 25 to 80 °C	[45]
9	Egg phosphatidylcholine and cholesteryl hemisuccinate	Liposome	These liposomes exhibited excellent stability at pH 7.4 and underwent rapid destabilization upon acidification	[14]
Thermal responsive carriers				
10	Poly(2-cinnamoylethyl methacrylate)-*block*-poly (*N-isopropylacrylamide*) (PCEMA-*b*-PNIPAM)	Vesicles	Thermosensitive release of drug 4-aminopyridine from the vesicles	[111]
11	Poly(ethylene glycol)-b-poly(N-isopropylacrylamide)	Vesicles	Doxorubicin-containing PEG–PNIPAM based vesicles, temperature induced transition allowed vesicle to release the drug	[56]
12	Poly(lactide)-b-poly(N-isopropylacrylamide)	Vesicles	Thermosensitive shell-cross-linked vesicles	[112]

13	Conjugated linoleic acid-coupled Pluronic F-127	Gel	For treatment of peritoneal metastasis of gastric cancer, Docetaxel was loaded into the gel	[57]
14	Poly(lactic acid-*co*-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-c*o*-glycolic acid)	Injectable sol–gel formulation	Long-acting injectable formulation in treatment of type II diabetes, degradation of the polypeptide drug exenatide was reduced	[62]
15	Carboxymethyl hexanoyl Chitosan with glycerophosphate di-sodium salt and glycerol	Injectable nanogel	Loaded with hydrophilic anti-epilepsy drug ethosuximide	[64]
16	Chitosan β-glycerophosphate (β-GP) gel	Gel	Thermo-gelation was observed above 33 °C, for the treatment of Critical limb ischaemia	[113]
17	Polybenzofulvene derivative (poly-6-MOEG-9-BF3k)	Nano-aggregates	Sustained release of leuprolide was observed at 37 °C while at lower temperature burst release pattern was observed	[114]
Magnetically responsive carriers				
18	Polybutadiene-b*lock*-poly(glutamic acid)	Nano-composites	Magnetic nanocomposites of polybutadiene-b*lock*-poly(glutamic acid) combined with hydrophobically modified γ-Fe_2O_3 nanoparticles.	[78]
			Structurally found as filled micelles or hollow vesicles with a magnetic membrane	
19	Poly(ethylene glycol)-b-poly(2-vinylpyridine)/ Poly(ethylene glycol)-b-poly(isoprene)	Nanoparticles	Magnetic nanoparticles have been assembled into the bilayer membrane of block copolymer to form oligo-lamellar vesicles	[115]

10.3.2 Applications

10.3.2.1 Anti Cancer Therapy

pH sensitive liposomes made of dioleoylphosphatidylethanolamine (DOPE) and oleic acid or DOPE and 1,2-dipalmitoylsuccinylglycerol explored multiple possibilities to treat cancer. Problems associated with less circulation half-life, stability in blood have been resolved [21]. Recently, liposomal delivery system modified with pH-responsive cell penetrating peptide TH (TH-Lip) has been reported. TH was found to be a wonderful pH responsive ligand as the cell penetrating capacity of TH concealed during the blood circulation and in normal tissues at neutral pH. However, when TH-Lip reached the tumour, low pH at these sites promoted protonation of TH and the surface charge of TH-Lip converted from negative to positive thus promoted enhanced cellular and tumour spheroid uptake [22].

10.3.2.2 Antibacterial Therapy

Bacterial infections are generally characterised by very low pH values because of anaerobic fermentation and subsequent inflammation. In this regard, systemic antibiotic therapy was achieved by incorporating an ionisable polyhistidine segment in a block copolymer to make PLGA-b-polyhistidine-b-PEG triblock copolymer nanoparticles. A charge switch at the sites of localised acidity promoted interactions with the negatively charged bacterial wall, and led to increased nanoparticle uptake in both Gram-positive and Gram-negative bacteria [23].

10.3.2.3 Intracellular Trafficking

The usefulness of pH-sensitive liposomes has been well exhibited in a wide variety of applications, these include the transport of fluorescent probes to estimate the efficacy of different liposome compositions and to explain the mechanisms involved in intracellular trafficking, the intracellular transport of antigens, targeting intracellular pathways involved in processing and presentation of antigens and enhancing the immune response to tumour cells [24].

10.3.2.4 Oral Bioavailability Improvement

Because of the broad range of pH found throughout the gastrointestinal tract, pH-responsive systems for oral drug delivery have been designed to protect drugs from the harsh conditions found in the gastric cavity and to improve their absorption in the intestine [25]. For instance, poly(methacrylic acid)-based copolymers have been used as pH-sensitive coatings at the surface of porous silica nanoparticles, as well as to prepare copolymer micelles able to disassemble at the intestinal pH [26].

This charge-reversal approach was also applied to multi stimuli responsive nanocarriers to achieve drug release at neutral pH by taking advantage of electrostatic interactions, and to chitosan nanoparticles for gastric or intestinal delivery [27]. Similarly it was reported, pH sensitive oly(methacrylic acid and methacrylate) nanoparticles improved the oral bioavailability of cyclosporin [18].

10.3.2.5 DNA Therapeutics

Gene therapy is most widely explored field in biomedical research and increasing interest in stimuli responsive carriers to deliver DNA therapeutics can open multiple opportunities in this area. pH senstive liposomes were developed to deliver plasmid DNA into mammalian cell lines [28].

10.4 Thermoresponsive Carriers

Thermoresponsive carriers are usually governed by a nonlinear sharp change in the properties of at least one component of the nanocarrier materials with temperature (Fig. 10.2 and Table 10.1). Such a sharp response triggers the release of the drug following a variation in the surrounding temperature. Ideally, thermosensitive nanocarriers should retain their load at body temperature (~37 °C), and rapidly deliver the drug within a locally heated tumour (~40–42 °C) to counteract rapid blood-passage time and washout from the tumour [2]. The use of temperature as a signal has been justified by the fact that the actual body temperature often deviates from the physiological value (37 °C) in the presence of pathogens or pyrogens. This deviation can be a useful stimulus to activate release of therapeutic agents from various temperature-responsive drug delivery systems for diseases accompanied by fever. Drug-delivery systems responsive to temperature utilize various polymer properties, including the thermally reversible transition of polymer molecules, swelling change of networks, glass transition and crystalline melting [29].

Thermoresponsive polymers utilize subtle changes in temperature to trigger macroscopic changes in material properties. Polymers that possess a lower critical solution temperature (LCST) typically undergo a sol-gel phase transition when heated above their LCST, whereas polymers that become soluble upon heating are said to possess an upper critical solution temperature (UCST) [6]. Both systems can be exploited for drug delivery purposes. LCST copolymers can simply be mixed with drug as a liquid suspension at room temperature and delivered via minimally invasive injection techniques directly to hard-to-access target tissues within the body. Heating to physiologic temperature drives a sol–gel phase transition, which entraps the infused drug within a solid depot and can provide sustained release of therapeutic concentrations of drug directly at the site of interest [30]. Drug-releasing polymer systems possessing a UCST may employ temperature-induced swelling or scaffold destabilisation to rapidly release drug at a target site [31]. Localised heating (tumour tissues) or the application of an externally applied

stimulus (ultrasound, infrared laser and so on) may be utilised to induce the local destabilisation of a UCST drug-releasing copolymer scaffold to produce targeted release [32, 33].

Typical LCST polymers are based on *N*-isopropylacrylamide (NIPAM), *N*,*N-diethylacrylamide* (DEA), methylvinylether and *N*-vinylcaprolactam (NVCL) as monomers. Some example of these categories are Poly(*N*-vinylcaprolactam) [34], Poly(*N*-isopropylacrylamide) [35], Poly(*N*,*N*-ethylmethylacrylamide) [36], Poly(*N-ethylacrylamide*) [37], Poly(*N*,*N*-diethylacrylamide) [38]. A typical UCST system is based on a combination of acrylamide and acrylic acid [6]. Thermoresponsiveness can also occur on a brief temperature decrease (also called cold shock or cryotherapy). In this case, a thermally reversible swelling or de-swelling of the nanocarrier leads to free diffusion of the encapsulated drugs as a consequence of increased porosity [39]. Thermosensitive amphiphilic polymers generally have temperature-responsive hydrophilic segments and a suitable hydrophobic segment. NIPAM and its random copolymers are the most intensively investigated temperature-sensitive hydrophilic segments [40]. Block copolymers of PEG as a hydrophilic block and NIPAM or poly(*N*-isopropylacrylamide)-co-*N*-(2-hydroxypropyl) methacrylamide-dilactate as a thermosensitive block are able to self-assemble in water into temperature-responsive nanocarriers above the LCST of the thermosensitive block [41]. The hydrophobic segments, poly (L-lactide), cholic acid, alkyl, and poly (γ-benzyl L-glutamate) have also been used in diblock polymers with the temperature-sensitive polyacrylamide derivatives being the hydrophilic segments.

10.4.1 Delivery Systems

Generally used thermoresponsive carriers are liposomes, or polymer micelles, nanoparticles and nanofibres [42–48]. For liposomes, thermoresponsiveness usually arises from a phase transition of the constituent lipids and the associated conformational variations in the lipid bilayer [49]. In vivo, heat is generally applied by using temperature-controlled water sacks, radiofrequency oscillators or miniature annular-phased array microwave applicators. Liposome-embedded hydrogels have been widely used for controlled drug release. Liu et al. embedded egg phosphatidylcholine liposome into a poly(*N*-isopropylacrylamide) (pNIPAM) hydrogel via chemical cross-linking. It was found that the confinement of the network and the hydrophobic interactions between the liposome and pNIPAM modulated the integrity of the liposome and the release profile of the encapsulated content, such as calcein [50].

Polymeric micelles have been explored for temperature induced release of actives for drug and gene delivery. The temperature-sensitive property is possessed by the outer shell of the polymeric micelles and the drug molecules are incorporated into the hydrophobic inner core [1]. Co-polymer of pNIPAM and poly(acrylic acid) with LCST 33 °C had shown potential to be developed as novel injectable drug delivery system due to rapid sol to gel conversion upon subcutaneous injection [51].

Chang et al. developed a block co-polymer from pNIPAM and poly(methyl methacrylate). They prepared prednisone acetate loaded uncross-linked micelles and cross-linked micelles with newly developed block co-polymer. LCST of uncross-linked and cross-linked micelles were 31.0 and 40.8 °C respectively. Uncross-linked micelles showed a rapid drug release near to 30 °C while cross-linked (SCL) micelles displayed negligible release up to 37 °C which increased rapidly above 40 °C [52]. Thermoresponsive, self-assembling polymersomes of poly(*N*-[3-aminopropyl] methacrylamide hydrochloride) and pNIPAM were also synthesised and used for similar applications [53].

Vitamin B-12 loaded nanofibres of pNIPAM/poly(ethylene oxide) (PEO) blend were also able to program drug release with the variation of temperature. Fibres containing higher ratios of pNIPAM displayed rapid release below LCST while the prolonged release was observed at 37 °C [54]. Stover and coworkers developed thermoresponsive, biodegradable linear-dendritic nanoparticles for targeted and sustained release of a pro-apoptotic drug ceramide (C6). These nanoparticles showed preferential uptake into human MDA-MB-231 breast adenocarcinoma cells at temperature above the LCST (37 °C) and sustained release of C6 up to 1 month in vitro [55].

10.4.2 Applications

10.4.2.1 Cancer Therapy

Thermoresponsive drug delivery is among the most investigated stimuli-responsive strategies, and has been widely explored in oncology. Qin et al. prepared thermoresponsive, doxorubicin-containing PEG–pNIPAM based polymersomes. Temperature induced transition facilitated self-assembly of polymer into vesicles at temperatures above 32 °C. Temperature-controlled release was determined by incorporating a hydrophobic fluorescent dye into their membrane. These vesicles destabilised, or ruptured upon local cooling with either ice or penetrating cryoprobes [56]. For the treatment of gastric cancer, linoleic acid-coupled Pluronic F-127 (Plu-CLA) based thermoresponsive hydrogel loaded with docetaxel were developed. Docetaxel–Plu-CLA showed excellent anti-tumour activity, induced apoptosis and significantly reduced the number of peritoneal metastatic nodules than docetaxel alone [57].

10.4.2.2 Anti-Adhesive

Another application of thermosensitive polymers to inhibit ischemia-induced postoperative peritoneal adhesion was highlighted by Wu and coworkers. The PCL–PEG–PCL, developed for this purpose exhibited rapid micelle formation at 10 °C and sol to gel conversion at body temperature. They were found to be well tolerated, less toxic and therapeutically more effective as compared to control

group. Emergence of a layer of neo-mesothelial cells on the injured tissues after micelle treatment provides a strong evidence in the support of its anti-adhesion activity [58].

10.4.2.3 Temperature-Responsive Surfaces

Temperature-responsive cell culture surfaces of pNIPAM with the ability to alter its surface hydrophobicity in response to temperature were developed. The so developed cell culture surfaces facilitated cell adhesion and proliferation at 37 °C while released spread cultured cells below 32 °C without any need of trypsin. Further, pre-coating of these surfaces with fibronectin improved spreading of less adhesive cultured hepatocytes [59]. Liao et al. developed NIPAM-based thermoresponsive polyelectrolyte multilayer films as culture substrates to support hMSC expansion. These film were via layer-by-layer adsorption of thermoresponsive polymer and positively charged allylamine hydrochloride, or negatively charged styrene sulfonic acid. Surface charge was found to alter ECM structure and subsequently cellular response for the surface. The positively charged surfaces resulted improved cell adhesion and growth compared to control surfaces [60].

10.4.2.4 Diabetes Mellitus

For treatment of diabetes mellitus, pancreatic islet cells were harvested on laminin-5 coated temperature-responsive dishes functional activity of the islet cell sheets was confirmed by histological examination and Insulin secretion assay prior to in vivo transplantation [61]. Thermoreversible hydrogel composed of poly(lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) triblock copolymers loaded with exenatide were also evaluated to treat diabetes. Polymer decreased the degradation of the polypeptide. Further, the problems of loading of water soluble peptide and sustaining the release of peptide were solved by synergistic effect of zinc acetate, PEG, and sucrose [62].

10.4.2.5 Ocular Therapy

Thermosensitive poly(*N*-isopropylacrylamide)–chitosan (pNIPAM–CS) solution loaded with timolol maleate was investigated for ocular application due to its in situ gel-forming properties. Polymer showed lower critical solution temperature of 32 °C, which was close to the surface temperature of the eye. Ocular pharmacokinetic analysis on rabbit eye showed higher Cmax and AUC as compared to conventional eye drop solution. Moreover, pNIPAM–CS solution showed reduced in vitro cytotoxicity and higher capacity to reduce the intra-ocular pressure as compared to conventional solution [63]

10.4.2.6 CNS Disorders

Thermo-gelling injectable nanogels amphiphilically modified chitosan were reported for delivery ethosuximide. In vivo studies suggested prominent therapeutic effect of ethosuximide loaded nanogels by suppressing spike wave discharges in Long Evan rat model [64].

10.5 Magnetically Responsive Carriers

A magnetic field-responsive nanocarrier generally involves paramagnetic or superparamagnetic materials either embedded into a polymeric scaffold forming liposomal, micellar, or supramolecular aggregates (Fig.10.2 and Table 10.1). The versatile intrinsic properties of magnetic particles enable their use in numerous medical applications, such as: localisation of therapy, where magnetic carriers, associated with drugs, nucleic acids or loaded within cells can be directed or guided by means of a magnetic field gradient towards certain biological targets; magnetic fluid hyperthermia, where selective thermal ablation of tumours is achieved through heating of tumour-localised magnetic particles exposed to a high frequency magnetic field; tissue engineering, where particles can be used in remote actuation for control of cellular behaviour enabling development of functional tissue or to provide means for a patterned cell assembly and facilitated seeding of tissue engineered scaffold with functional cells; and MRI, where magnetic particles are used as contrast agents [65].

Magnetic systems for magnetic targeting that have been proposed or employed so far fall into two main classes. In one class, magnets external to the body provide both the field to magnetize the carrier and field gradients for targeting [66, 67]. However, the use of external magnets imposes serious limitations in targeting deep tissues as their field strength and field gradient decrease exponentially with the distance from the surface. The other class is based on a combination of external magnets and magnets (or magnetizable devices) implanted local to the target region. In the second class of systems, the external magnet would typically provide the magnetizing field for the carrier, while the local magnet (or magnetisable implant) will provide the largest possible field gradients for targeting. The second type of magnet system can be of potential use for targeting deep tissues, including blood vessels where magnetizable implants can be placed [68–70]. The effective use of magnetically responsive nanocarriers for biomedical applications such as targeted drug delivery depends on a number of factors related to the size and magnetism of the biocompatible nanoparticles. Parameters such as the physicochemical properties of the drug-loaded nanocarriers, field strength and geometry, depth of the target tissue, rate of blood flow, and vascular supply play a role in determining the effectiveness of this method of drug delivery [71, 72].

Iron oxides with core/shell structures are the most widely used as sources of magnetic materials. Iron oxides have several crystalline polymorphs known as α-Fe_2O_3 (hematite), β-Fe_2O_3, γ-Fe_2O_3 (maghemite), ε-Fe_2O_3, Fe_3O_4 (magnetite) and some others (amorphous and high pressure forms). Nevertheless, only maghemite and magnetite found the greatest interest of bio-applications [73]. Readily, carbonyl iron, which is well-known material with a unique form of elemental iron because of its small particle size, was also used as magnetic core [74]. In some reports, pure metals, such as Fe and Co were chosen as a magnetic material because they have several advantages over iron oxides, e.g. better magnetic properties, high saturation magnetisation, and high specific loss of power [75, 76].

Functionalisation of magnetically responsive carriers with amino group, silica, polymer, various surfactants or other organic compounds is usually provided in order to achieve better physical and chemical properties. Moreover, the core/shell structures of nanocarriers have the advantages of good dispersion, high stability against oxidation and appreciable amount of drug can be loaded to the polymer shell [77]. Lecommandoux et al. developed magnetic nanocomposites of polypeptide-based diblock copolymers of polybutadiene-block-poly(glutamic acid) in combination with hydrophobically modified γ-Fe_2O_3 nanoparticles [78]. Furthermore, lots of functional groups from polymers on the surface can be used for further functionalisation to get various properties [79]. It is favoured that magnetically responsive carriers retain sufficient hydrophilicity and, with coating, do not exceed 100 nm in size to avoid rapid clearance by reticuloendothelial system. It was found that surface functionalisation also plays the key role in nanoparticle toxicity [80].

10.5.1 Delivery Systems

Candidate nanosystems for such a therapeutic approach are core–shell nanoparticles (a magnetic core made of magnetite coated with silica or polymer) [81, 82], magneto-liposomes (maghemite nanocrystals encapsulated in liposomes) [83, 84] and porous metallic nanocapsules [85] (Fig. 10.2). A novel nanocarrier, containing functionalised magnetite (Fe_3O_4) core that was conjugated with drug via acid-labile hydrazone-bond and encapsulated by the thermosensitive chitosan-g-poly(*N-isopropylacrylamide-co-N*,*N*-dimethylacrylamide) was reported. Polymer exhibited a LCST of 38 °C below which the drug release response was appreciably low [86]. Antibody-conjugated magnetoliposomes for targeting cancer cells were also reported [87].

10.5.2 Applications

Magnetically responsive carriers are getting significant attention in the field of theranostics. Theranostics is the fusion of therapeutics and diagnostics to design individualised pharmacotherapy. Paramagnetic nanoparticles were initially used as contrast agents for magnetic resonance imaging (MRI) later on surface modification

of these nanoparticles introduced various functions for the nanoparticles to be used for both gene delivery and MR imaging. The combination based on the nanoparticles allows non-invasive monitoring of in vivo gene delivery with MRI and delivery of therapeutic genes [88, 89]. Such magnetofection experiments were generally performed using nanoassemblies with cationic coatings to condense nucleic acids, which resulted in higher transfection efficiencies under a permanent magnetic field. These technique led to improved effectiveness in the transfection of siRNA in vitro and/or in vivo when directed against prostate [90] and breast cancers [91], as well as in the gene transfer to oligodendrocyte precursors for neural repair [92].

10.6 Recent Advancements

10.6.1 Dual and Multi Stimuli Responsive Carriers

In an effort to further fine tune drug release and augment therapeutic efficacy of nanoparticulate drugs, sophisticated polymeric nanoparticles that respond to dual and multi-stimuli such as pH/temperature, pH/redox, pH/magnetic field, temperature/reduction, double pH, pH and diols, temperature/magnetic field, temperature/enzyme, temperature/pH/redox, temperature/pH/magnetic, pH/redox/magnetic, temperature/redox/guest molecules and temperature/pH/guest molecules have been aggressively pursued (Fig. 10.2) [93–96]. It should be noted that the responses take place either simultaneously at the same location or in a sequential manner in different settings and/or compartments. These dual and multi-stimuli responsive polymeric nanoparticles might on one hand offer unprecedented control over drug delivery and release leading to superior in vitro and/or in vivo anti-cancer potency, and on the other hand also facilitate nanoparticles preparation and loading of drugs under mild conditions [97]. These two and more stimuli are combined in order to: (1) facilitate preparation of nanoparticles under mild conditions through application of an external stimulus such as temperature and pH; (2) trigger drug release via application of an external stimulus such as magnetic field, ultrasonic, light and temperature; (3) trigger drug release or reverse deshielding of nanoparticles thereby enhancing tumour cell uptake of nanoparticulate drugs in a mildly acidic tumour microenvironment; and/or (4) boost intracellular drug release in tumour cells under endo/lysosomal pH and/or cytosolic reductive conditions.

Shim et al. developed a polymer containing sulfamethazine as the pH-responsive component, and poly(ε-caprolactone-co-lactide) (PCLA) in a triblock with PEG, PCLA–PEG–PCLA, as the thermosensitive moiety [98]. By controlling precise ratios between the two parts the co-polymer showed a reversible sol–gel–sol transition phase. At room temperature and pH 8 the polymer remained in a solution state, when the environment was altered to 37 °C and pH 7.4, i.e. normal physiological conditions, there was a rapid phase transition to a gel state. Once the gel was formed it remained stable and degraded over time without changing local pH levels [98, 99]. Despite the advantageous versatility of these systems, they often appear as too

Table 10.2 Clinical trials for thermal and magnetically responsive carriers

S.No.	Clinical trial	Indication	Carrier	Drug	NCT no.	Phase	Status
Thermal responsive carriers							
1	Phase 1/2 study of ThermoDox with approved hyperthermia in treatment of breast cancer recurrence at the chest wall	Breast cancer	Liposome	Doxorubicin	NCT00826085	I/II	Recruiting
2	A study of ThermoDox™ in combination with radiofrequency ablation (RFA) in primary and metastatic tumors of the liver	Hepatic and liver neoplasm	Liposome	Doxorubicin	NCT00441376	I	Completed
3	Phase 2 study of Thermodox as adjuvant therapy with thermal ablation (RFA) in treatment of metastatic colorectal cancer(mCRC) (ABLATE)	Colon cancer Liver metastasis	Liposome	Doxorubicin	NCT01464593	II	Terminated
4	Study of ThermoDox with standardized radiofrequency ablation (RFA) for treatment of hepatocellular carcinoma (HCC) (OPTIMA)	Hepatocellular carcinoma	Liposome	Doxorubicin	NCT02112656	III	Not started
5	MRI guided high intensity focused ultrasound (HIFU) and ThermoDox for palliation of painful bone metastases	Bone metastasis, breast and lung cancer	Liposome	Doxorubicin	NCT01640847	II	Not started
6	Phase 3 study of ThermoDox with radiofrequency ablation (RFA) in treatment of hepatocellular carcinoma (HCC)	Hepatocellular carcinoma	Liposome	Doxorubicin	NCT00617981	III	Ongoing
7	Temperature-sensitive liposomal doxorubicin and hyperthermia in treating women with locally recurrent breast cancer	Breast cancer	Liposome	Doxorubicin	NCT00346229	I	Terminated
8	Heat activated liposomal doxorubicin and radiofrequency ablation in treating patients with primary or metastatic liver tumors	Liver cancer, metastatic cancer	Liposome	Doxorubicin	NCT00093444	I	Not completed
Magnetically responsive carriers							
9	Ferumoxytol—Iron oxide nanoparticle magnetic resonance dynamic contrast enhanced MRI	Head and neck cancer	Iron oxide nanoparticle	Ferumoxytol	NCT01895829	0	Ongoing
10	Magnetic-targeted doxorubicin in treating patients with cancer metastatic to the liver	Cancer	Iron and carbon magnetic beads	Doxorubicin	NCT00041808	I/II	Completed

11	Safety and efficacy of doxorubicin adsorbed to magnetic beads	Hepatocellular carcinoma	Iron and carbon magnetic beads	Doxorubucin	NCT00054951	I/II	Unknown
12	Safety and efficacy of doxorubicin adsorbed to magnetic beads vs. iv doxorubicin in treating liver cancer	Hepatocellular carcinoma	Iron and carbon magnetic beads	Doxorubucin	NCT00034333	II/III	Terminated
13	Magnetic nanoparticle thermoablation-retention and maintenance in the prostate:a phase 0 study in men (MAGNABLATE I)	Prostate cancer	Magnetic nanoparticle	–	NCT02033447	0	Not started
14	High-field MRI iron-based contrast-enhanced characterization of multiple sclerosis and demyelinating diseases	Multiple sclerosis	Iron oxide nanoparticle	Feraheme Gadolinium	NCT01973517	–	Not started
15	Pre-operative nodal staging of thyroid cancer using ultra-small superparamagnetic iron oxidemagnetic resonance imaging (USPIO MRI): preliminary study	Metastatic medullary thyroid cancer	Iron oxide nanoparticle	Ferumoxytol	NCT01927887	–	Recruiting
16	Plasmonic photothermal and stem cell therapy of atherosclerosis versus stenting (NANOM PCI)	Coronary artery disease	Gold nano-particles with iron oxide-silica shells	–	NCT01436123	I	Completed
17	USPIO magnetic resonance imaging (MRI)	Cancer of lymph node	Iron oxide nanoparticle	Feraheme®	NCT01815333	–	Recruiting
18	Iron nanoparticle enhanced MRI in the assessment of myocardial infarction (IRNMAN)	Myocardial infarction (MI), Inflammation	Iron oxide nanoparticle	Ferumoxytol	NCT01995799	II	Recruiting
19	Pre-operative staging of pancreatic cancer using superparamagnetic iron oxide magnetic resonance imaging (SPIO MRI)	Pancreatic cancer	Iron oxide nanoparticle	Feraheme®	NCT00920023	IV	Ongoing
20	A validation study of mr lymphangiography using SPIO, a new lymphotropic superparamagnetic nanoparticle contrast	Bladder, Genito-urinary, prostate cancer	Iron oxide nanoparticle	Feraheme®	NCT00147238	–	Terminated
21	Ferumoxytol for magnetic resonance imaging of myocardial infarction	MI	Iron oxide nanoparticle	Ferumoxytol	NCT01323296	–	Unknown
22	Inflammatory cell trafficking after myocardial infarction	MI, inflammation	Iron oxide nanoparticle	–	NCT01127113	–	Unknown

complicated and many still remain as proofs of concept. To ascertain the viability of these strategies, evidence of the regulation of the response to each stimulus would be needed both in vitro and in vivo.

10.6.2 Breathing Vesicles

The breathing, in this context, can be defined as a highly reversible vesicle volume change by a factor of approximately 7, which was accompanied by diffusion of species into and out of the vesicles with a relaxation time of approximately 1 min. A three-layered vesicle system with pH-induced "breathing" feature was designed with triblock copolymer poly(ethylene oxide)-block-polystyrene-block-poly (2-diethylaminoethylmethacrylate). Self-assembly into vesicles was observed at a pH of 10.4. As the pH decreased, both the vesicle size and the thickness of all three layers increased. Progressive swelling of the middle layer with a decrease in pH below 6 induced cracking of the two outer layers and also a sharp increase of the vesicle size and the wall thickness. When pH reached up to 3.4, the vesicle size was found to be increased by a factor of 1.9 and the wall showed a cracked surface. These changes between pH 10.4 and 3.4 were highly reversible with the relaxation time of 1 min with marked repeatedly. The change in the wall structure dramatically helped to increase the wall permeability to water along with rate of proton diffusion from practically zero to extremely rapid [100].

Similarly, CO_2-responsive breathing vesicles were synthesised with block polymer poly(ethylene oxide)-poly(*N*-amidino)dodecyl acrylamide (PEO-b-PAD). PEO-b-PAD self-assembled in to micelle like structure in which the PEO portion formed hydrophilic exterior and the PAD portion formed hydrophobic interior. The amidine group in copolymer transformed into a charged amidinium species upon reaction with CO_2 which reverted back to its original form upon exposure to argon (Ar). It was confirmed by much larger intact vesicles with a diameter of 205.25 nm and strikingly increases volume by 83.5 % after CO_2 treatment for 20 min. These vesicles shrunk back to their initial size in the presence of Ar [101].

10.7 Challenges

Despite their responsive feature and versatility for drug delivery, thermoresponsive systems have some unanswered questions too. Co-polymeric systems provided ease to modulate the system properties but they are facing the problem of biocompatibility, biodegradability, reproducibility and tailored drug release as per requirement of site of action [51, 99]. Development of amphiphilic poly(asparagine) based polymers, Poly[α/β-(DL-aspartate isopropylamide)-co-(succinimide)] PCL-PEG copolymer had solved the biodegradability issue up to certain extent still, more in vivo studies are needed to ascertain their complete safety [58, 102–104].

While theranostics is the most recent advancement which utilizes the magnet stimuli as a component of diagnosis still the mismatch of dose required for imaging and therapy creates problem and require further optimisation. Further, the concentration required for imaging may create toxicity problems [105].

10.8 Clinical Status

Nanocarriers that are responsive to exogenous stimuli (temperature and magnetic field) have reached clinical stage as they are more promising. Endogenous triggers are indeed difficult to control because they may vary from one patient to another (such as the pH of a tumour or the presence of reducing agents in the blood circulation). Table 10.2 gives information regarding responsive carriers that are in different phases of clinical trials.

10.9 Conclusions

With greater understanding of physiological differences between normal and disease tissues and advances in material design, there is an opportunity to develop nanocarrier systems for target-specific drug delivery that will respond to local stimuli. They can shift the paradigm in the delivery of medicine and diagnostics. This chapter explained the role of pH, temperature and magnetic field responsive nanocarriers for targeted-drug delivery. However, compared to the amount of research being done in the field, relatively few medical nanotechnologies have made it to the market. Clear demonstrations of biocompatibility and including biodegradable components will make these materials even more attractive for in vivo applications. Furthermore, development and implementation of scalable, cost-effective fabrication techniques will help promote clinical translation. Together, intelligent materials and nanocarriers provide a versatile toolbox that we believe will revolutionize the future of modern medicine.

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