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Stimulus-Responsive Sequential Release Systems for Drug and Gene Delivery

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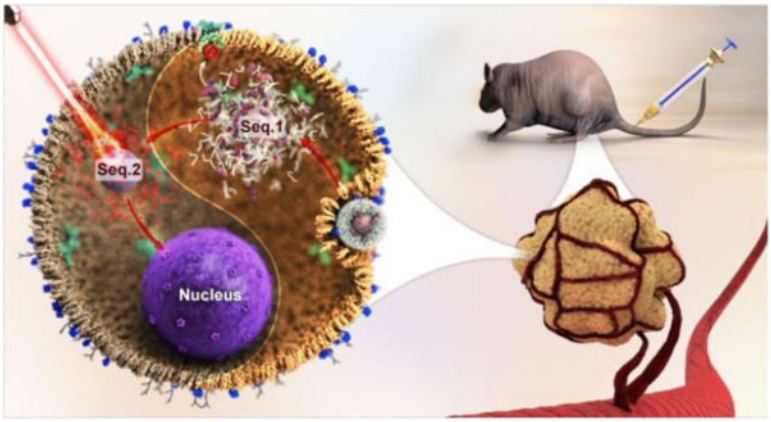
Abstract

In recent years, a range of studies have been conducted with the aim to design and characterize delivery systems that are able to release multiple therapeutic agents in controlled and programmed temporal sequences, or with spatial resolution inside the body. This sequential release occurs in response to different stimuli, including changes in pH, redox potential, enzyme activity, temperature gradients, light irradiation, and by applying external magnetic and electrical fields. Sequential release (SR)-based delivery systems, are often based on a range of different micro- or nanocarriers and may offer a silver bullet in the battle against various diseases, such as cancer. Their distinctive characteristic is the ability to release one or more drugs (or release drugs along with genes) in a controlled sequence at different times or at different sites. This approach can lengthen gene expression periods, reduce the side effects of drugs, enhance the efficacy of drugs, and induce an anti-proliferative effect on cancer cells due to the synergistic effects of genes and drugs. The key objective of this review is to summarize recent progress in SR-based drug/gene delivery systems for cancer and other diseases.

Graphical abstract



Keywords

sequential drug and gene release; stimulus-responsive nanoparticles; cancer nanomedicine; temporal control; synergistic combinations

1. Introduction

Nanotechnology is concerned with the design and development of materials with dimensions ranging from approximately 1 nm to even hundreds of nanometers, which enables the design and fabrication of materials with a defined structural molecular architecture. These materials possess improved and tunable, optical, electrical, chemical, physical and biomedical properties. Nanotechnology has provided an effective platform for smart drug and gene delivery systems, and has led to the development of many innovative materials for the safe delivery and on-demand release of a wide variety of therapeutic agents into specifically targeted cells and tissues [1–3]. The term “smart” refers to the ability of drug/gene delivery systems (DGDSs) to provide controlled release of the cargo in the exact time and place required in response to rationally chosen stimuli which may be external or internal [4]. If the cargo is released at an inappropriate time or place, not only does this restrict the efficacy of DGDSs, but it also limits the choice of delivery routes for administering proteins, nucleic acids or drugs, where the oral delivery route would be much preferred. The pharmaceutical and/or genetic cargos incorporated inside the nanocarriers require effective protection and on-demand release, or else they could end up being degraded in the wrong cellular location and/or in the stomach acidic environment. Hence, an ideal carrier should fulfill the following two requirements at the same time; firstly, protecting the cargo from being released until it reaches the desired site, and secondly, being designed to be degraded or disrupted itself when it does reach the targeted tissue, in order to deliver the cargo with the highest possible local concentration [5, 6].

Nanoparticles (NPs) are often selected as drug delivery systems and can be chosen from a broad range of different nanomaterials such as (1) inorganic NPs *e.g.,* gold NPs (AuNPs), mesoporous silica NPs (MSNs), or magnetic NPs (MNPs) and (2) organic NPs e.g. dendrimers, liposomes, and polymeric NPs [7, 8]. Due to their small size, good biocompatibility, easy surface modification as well as the other considerable surface properties, these NPs have so far shown great promise to function as smart DGDSs. They also possess a longer blood circulation time, and a higher surface to volume ratio, along with reduced levels of toxicity [9, 10]. Smart NPs in the form of stimulus-sensitive NPs stand out, for their sensitivity depends on the inherent environmental conditions of tumor cells or tissues, as well as the judicious application of externally applied physical triggers. Stimulus-responsive nanocarriers can be categorized into two groups; internal stimulus-responsive nanocarriers (pH, redox, and enzyme activity) and external stimulus-responsive nanocarriers (light, magnetic, electric fields, and temperature gradients) [4, 11].

Inorganic nanoparticles (iONPs), as well as organic nanoparticles (ONPs), are synthesized using chemical techniques. The biocompatibility of NPs is grounded in their unique structure and surface properties. It is also possible to increase the biocompatibility of some NPs such as MNPs, biopolymers, and micelles through attaching additional silica-based nanomaterials [12–15]. Liposomes are phospholipid bilayer vesicles containing two different environments (hydrophobic and hydrophilic), ideal for the simultaneous delivery of hydrophobic and hydrophilic molecules that can be either drugs or genes [16, 17]. Another effective group of carriers for SR-based delivery systems are dendrimers, which have a defined three-dimensional (3D) structure, enabling modification of their properties through

tailoring the chemical groups on their surface. Polymers can be biodegradable and can allow drug release and effective protection of genes and drugs while being themselves perfectly degradable [18]. Janus NPs can possess two or more different physical properties in different regions of the same particle [19]. Layer-by-layer and core-shell particles can enable the co-delivery of multiple therapeutic agents incorporated in different layers [20, 21]. Along with NPs, a number of microparticles (MPs), ranging from 1 to 1000 μm in diameter, have also been used as stimulus-sensitive SR carriers. These MPs includes microcapsules and microspheres that can be able to deliver larger amounts of drugs than NPs, as well as having higher compatibility and bioavailability in some cases [22, 23].

Despite the rapid advances in DGDSs, the emergence of SR techniques has further energized the field by unlocking the potential of both organic and inorganic NPs as well as MPs, to deliver significant amounts of genes and drugs to the targeted tissues or even intracellular organelles while preventing the unintended release. SR-based DGDSs are designed to regulate the release of drugs/genes in a sequentially controlled manner following a programmed temporal or spatial sequence [24, 25]. The main distinguishing feature of these smart systems is to be able to respond to a variety of external stimuli such as temperature gradients, magnetic fields, light irradiation, and ultrasound, as well as internal stimuli (*e.g.,* pH variations, enzymatic activation, redox potential) [26]. SR-based DGDSs can deliver a combination of genes, drugs, or both at the same time, or with a specific sequential release order to improve the efficiency of therapeutics [27, 28]. Releasing multiple therapeutic agents in a sequential manner with temporal and spatial control may play a key role in increasing the effectiveness of cancer therapy because it is one way to tackle multidrug resistance (MDR) pumps in cancer cells. This is because the pump may be inactivated using a specific inhibitor first, and then the other chemotherapy drug(s) can be released, so as to enhance their performance in killing cancer cells without being blocked by the MDR effect. On the other hand, employing SR with one or more small interfering RNA (siRNA) genes can reduce gene expression (gene silencing) leading to deactivation of drug resistance genes, which makes the tumor cells more sensitive to drugs and improves the efficiency of chemotherapy [29]. In some cases, the sequential co-delivery of multiple drugs with different molecular targets, allows one drug to change the cancer cell metabolism, which opens up an opportunity for the other drug to induce apoptosis in tumors [30].

In recent years, a number of cutting-edge technologies have led to significant advancements in DDSs. The microarray technology, for instance, has been effective in the detection of new molecular and clinical targets [31, 32]. Arrays such as titania nanotube arrays allow the delivery of two or more hydrophilic and hydrophobic drugs with a controlled release pattern [33]. The introduction of 3D printers has laid the foundation for the fabrication of special 3D-modeled implantable devices containing nanocarriers, with remarkable accuracy and various shapes such as stents, tubes, etc. [34, 35]. Electro-spraying has so far shown great potential for the preparation of MPs and NPs for DDSs in just a single step, and a wide range of drugs can be encapsulated together while the particles still have a high surface area [36, 37].

In addition, SR also plays a key role in delivering growth factors and proteins, in regenerative medicine, wound healing, and angiogenesis. Generally, in tissue engineering or

angiogenesis, it is more effective to release multiple growth factors in a programmed sequence, because the processes of regeneration and angiogenesis take place in a number of sequential steps. Therefore, using all the growth factors at once (or using just a single growth factor) would not be as efficient as using SR. A considerable number of articles have been published regarding review of the synergistic and sequential drug delivery systems [38] and comparing SR both *in vitro* and *in vivo* [39], but the present review article aims to discuss the smart delivery systems specifically designed to function by SR mechanism. Fig. 1 illustrates the effect of these stimulating factors in allowing the sequential release of various therapeutic agents, especially in tumors. This review will investigate the latest studies that have employed various types of iONPs, ONPs, and MPs in their smart DGDSs, followed by the coverage of some useful technologies (*e.g.,* 3D-printer and arrays) and their effects on the SR of drugs or genes; ultimately concluded with a discussion about the advantages and limitations of these systems along with casting a critical eye over the issue.

2. Important of Types of Sequential Release Carriers

One important goal in designing controlled release systems is to maintain an appropriate drug concentration in the blood by screening and optimizing the levels of both effectiveness and toxicity. Drug release patterns from nanocarriers are governed by various factors such as the composition, ratio, and types of interaction between the carriers and the therapeutic agents. Mechanisms of drug release from carriers can be classified into diffusion, chemical reaction, dissolution, and stimulus-responsive release [40]. The administration of carriers loaded with drugs has shown success in overcoming traditional problems associated with the administration of single dosages of low molecular weight drugs that can have severe side effects. However, in severe cases of MDR cancer, the combination use of several chemotherapy drugs is required. Therefore, designing systems that can carry out SR of multiple drugs is a priority. Several strategies have been applied to overcome MDR including administration of Janus particles, layer-by-layer (LBL) modified particles and core-shelled structured systems. In the following sections, the mechanisms of action for each system are discussed and examples of these types are provided.

2.1. Janus particles

Janus particles which can have dimensions in the nanometer or micrometer range are distinct types of particle that can have two or more different physical properties at the same time. These types of NPs were named after the Roman god “Janus” who had two faces. The synthesis, classification, characteristics, and application of Janus NPs has been reviewed in the literature several times, but there is a lack of discussion about the role of Janus particles in the SR systems [41–44]. Some types of Janus NPs have found applications for SR, including dendrimers, micelles, polymeric NPs, polymer-liposome, and polymeric-inorganic hybrid structures, and all inorganic-based Janus NPs [45]. The basis for engineering these particles for SR relies on having two faces with distinct physicochemical features, such as hydrophilic and hydrophobic properties. Therefore, two therapeutic agents with different properties (hydrophilic or hydrophobic) could be loaded into different regions of the particle. Furthermore, to tailor the order of release, the systems can be designed in such a way as to respond to two different types of external stimulus. Except as mentioned, other types of

release mechanisms have been devised for SR Janus particles, which have been discussed afterward.

Polyethylene glycol (PEG)-based Janus dendrimers were explored for SR of model drugs; benzyl alcohol and 3-phenylpropionic acid were conjugated to dendrimers via carbonate or ester linkages. In this system, four types of PEG-based dendrons were connected via a 3+2 cycloaddition reaction (click reaction) between azide and alkyne groups in dendrons. The system was shown to be both biocompatible and hemocompatible [46]. Several studies have been performed on liposome/polymer [47] and polymer/polymer [48] combination particles, but the SR platform was not elaborated. A unique drug delivery system is capable of being applied for multimodal SR by using polymer/inorganic hybrid octopus-like Janus NPs. Gold NPs coated with polyacrylic acid were used as a template for mesoporous silica to form the basic Janus particle and modification with PEG were performed. The octopus-like assembly of NPs was applied as a targeting agent for photothermal and chemotherapy applications [49]. The heterogeneous structure of the inorganic Janus NPs allowed the loading of multiple therapeutic agents. The sequential release of hydrophobic docetaxel and hydrophilic doxorubicin (DOX) was obtained by a Janus type NP composed of a gold nanocage coated by poly(3-caprolactone) and $Fe(OH)_3$-poly acrylic acid. The SR was achieved by the release of DOX at low pH and the release of docetaxel after NIR laser irradiation. The entrapment of docetaxel in the gold nanocages allowed release by NIR irradiation, while DOX was entrapped in the polyacrylic acid layer, and the releasing mechanism was controlled by pH [19].

2.2. Layer-by-layer modified particles

Thin films that have been prepared using layer-by-layer (LBL) technology have been utilized in the construction of implantable devices [50, 51]. LBL assembled materials can provide spatially controlled and SR, while the bulk degradation of the LBL coating is inhibited [52]. The adsorption of bioactive agents into the LBL assembled structure could be designed according to the requirements of the multidrug delivery system. LBL films containing cationic poly(amidoamine) with a disulfide cross-linking agent containing DNA were prepared as a candidate for vaccine DNA delivery. To inhibit bulk degradation of the platform and leading to burst release, an additional poly(ethylenimine) layer was inserted onto the system. The whole complex was shown to be an effective bioreducible LBL with SR capability [52]. A similar LBL platform allowing sequential gene delivery was constructed of a bioreducible polymer containing poly(amidoamine), cystamine-bisacrylamide and aminopentanol [21].

2.3. Core-shell structures

Core-shell structures are another class of nanoplatform capable of integrating two or more functions via surface modification. Core-shell NPs have the shell built up upon the core, while alternatively the core can also be removed from the complex, leaving a hollow shell structure [53]. A synergistic combination of combretastatin A4 (CA4, an antivasculature agent) and DOX (an anticancer chemotherapeutic) was investigated using core-shell NPs to deliver to *in vitro* models of human umbilical vein endothelial cells (HUVECs) and melanoma cells B16-F10 [54]. In this system, coaxial electro-spraying was used to fabricate

poly(lactic-co-glycolic acid) (PLGA) as a shell onto a core consisting of one of two different polymers, hydrophilic polyvinylpyrrolidone (PVP) and hydrophobic poly(ε-caprolactone) (PCL). With over 90% encapsulation efficiencies, the core was loaded with CA4 while DOX was incorporated in the shell. The results of this study indicated that at neutral pH, the release profile of CA4 from PVP-DOX/PLGA-CA4 was faster than from PCL-DOX/PLGA-CA4. Both CA4 and DOX showed a decreased release profile from PCL-DOX/PLGA-CA4. The results from the cell cytotoxicity studies showed that B16-F10 and HUVECs cells were killed by the NPs in a dose-dependent manner. The expression of HIF1-α and VEGF was dramatically attenuated during the treatment.

Recently, another SR combination drug delivery approach was reported using hollow mesoporous silica NPs (HMSNs) [55]. In this system, DOX hydrochloride was loaded onto the surface of the HMSNs, and verapamil into the inner mesopores of the hollow silica NPs for treatment of human nasopharyngeal carcinoma cells (KB). The polymeric shell was degraded by the mechanism of acidic pH-dependent cleavage, generating positive charges and swelling of the NPs. These changes led to the internalization of the NPs into the tumor cells releasing the loaded drugs.

3. Sequential Release-based Delivery Systems

In the controlled release (CR) of a drug, the concentration of the drug rapidly reaches the required level of a predetermined and programmable concentration in the tissues or organs, and is maintained at that level for some time, thus reducing the side effects throughout the whole body. In some CR systems, it is even possible to tailor the actual rate of drug release. Selecting the most appropriate carrier for genetic and/or medicinal cargos, and determining the route that the loaded carrier should take to reach the intended tissue in the body are among the main requirements of smart delivery systems. In controlled release systems, defined amounts of drugs are released over a period of time in a repeatable manner. For instance, hyaluronic acid (HA)–gelatin-PEGylated functionalized MSNs (MSN@HA-gelatin-PEG) could provide the controlled and efficient release of DOX in breast cancer cells. These types of systems are called “multifunctional envelope type nanodevices” (MEND) and have found applicability for the design of smart DDS to overcome biological barriers. In this system, DOX was loaded after the formation of MSN@HA. When the particles reached the tumor site, in an enzyme responsive process (due to the high expression level of matrix metalloproteinases-2 (MMP-2) the gelatin layer was hydrolyzed and the cargo was deshielded. Furthermore, MSN@HA/DOX was trapped by cancer cells through HA receptor-mediated endocytosis and DOX was released via hyaluronidase-catalyzed degradation of HA [56]. The MEND strategy is the basis of MSN@HA-gelatin-PEG/DOX system, and a schematic illustration of this nanoplatform is presented in Figure 2.

The sequential drug/gene delivery systems, which are a subset of controlled drug delivery systems, offer the option of differential temporal or spatial control over the release of one or more active agents, and can lead to increasing the therapeutic efficiency [55, 57]. These stimulus-responsive SR-based DGDSs, also known as smart nanocarriers, show release mechanisms based on internal or external triggering factors, such as pH variation, redox potential, enzymatic activation, temperature gradient, light, ultrasound and also electrical

and magnetic fields [26]. Compared to normal tissues, tumor tissues display a number of changes in the intracellular microenvironment such as over-expressed reduced glutathione (GSH, 2–10 mM) [58] and lower pH (5.5–6.5). In light of these biological changes, new drug targeting methods have been developed, relying on a wide variety of engineered iONPs, ONPs, and MPs, which designed to be sensitive to changes in the presence of stimulus factors [59]. Temperature, for instance, is a useful biological stimulus because in case of infection there are obvious temperature changes specifically in the tissues, and this factor can be used as an external stimulus for triggering the release of genes or drugs [26, 60]. In sequential release systems, the carriers can be functionalized with two or more components that respond to multiple stimuli, in order to precisely release two or more -cargos at the target site at different times [61].

Recently, in a thematic issue of the journal of “Advanced Drug Delivery Reviews” Becker et al. and Chew et al. highlighted the aspects of sequential release in delivery systems [62, 63].

3.1. pH fluctuation

Several studies have so far been conducted, investigating DGDS systems specifically designed to provide SR-based on pH changes, and the fact that different tissues of the body (especially tumors) have different pH ranges. Normally, the physiological pH of the human body is around 7.4, ranging from 1.5 to 3.5 in the stomach, 5.0 to 6.0 in endosomes, and 4.0 to 5.0 in lysosomes, while the pH of cancerous tissues is reduced due to the Warburg effect which arises because the hypoxic cells produce lactic acid due to glycolysis [4, 64, 65]. Since the specific environment of tumor cells such as hypoxia and having an acidic pH gives rise to a number of problems and leads to limiting the activity of antitumor drugs, the introduction of pH-sensitive DGDSs can increase the therapeutic efficacy of drugs or genes with both maintaining the drug or gene from unwanted mechanisms and reactions to other tissues/organs or even biological and chemical compounds, and also releasing the drug and gene in a controlled and sequential manner which leads to performing a programmable medicinal schedule for a certain disease [66].

Different types of nanomaterials have been used to design pH-sensitive DGDSs, including iONPs (MSNs, AuNPs, and MNPs) [67, 68], liposomes [69], polymers [70, 71], hydrogels [72], and core-shell NPs [20]. Generally, pH-sensitive polymers, including weak acidic and basic groups that act as poly-electrolytes, are employed. These polymers can be derived from both natural sources (chitosan, gelatin, protein, starch, and cellulose derivatives) [73–77] or synthetic routes (polyanhydrides, α-hydroxy acids, esters, anhydrides, acetals, carbonates, amides, urethanes, phosphates, poly(D,L-lactic acid) (PDLLA), polylactic acid (PLA) (both L- and D, L-lactide forms), poly(glycolic acid) (PGA), and poly(lactic-co-glycolic acid) (PLGA)) [78, 79]. Numerous pH-sensitive polymeric NPs have been prepared including chitosan, polyanions, a mixture of them, and also cross-linked polymers (nanogels) [80, 81]. Carboxylic acid groups, which is counted to be a weak acid, act as proton donors in the synthesis of poly(propylacrylic acid) (PPAA), polyanions such as poly(acrylic acid) (PAA), poly(butyl acrylic acid) (PBAA), poly(methylacrylic acid) (PMAA), and poly(ethylacrylic acid) (PEAA) [4]. Polymeric NPs can also be formed using a mixture of polyanions and polycations, such as chitosan mixed with Eudragit [82].

Drug resistance is a major barrier to the success of cancer chemotherapy. Many factors are involved in the development of drug resistance by cancer cells, which can be partly overcome by inhibiting cellular autophagy, inducing apoptosis, or inhibiting the efflux pumps, P-glycoprotein, or multidrug resistance protein 1 (MDR1). A number of studies have employed MSNs to overcome MDR by the controlled release of drugs/genes [55, 83]. These inorganic nanocarriers, which can be obtained from both natural and synthetic sources having dimensions ranging from 50 nm to 300 nm, are of great interest in SR-based systems due to their special structures and biochemical properties. These properties include, large surface area, tunable particle/porous morphology, good stability, and the capability for control of the surface charge. The high loading capacity of MSNs allows the transfer of two or more drugs/genes simultaneously, and can be used for combination therapy [84–86]. The toxicity of MSNs has been found to depend on their surface functionalization. Increasing the biodegradability of MSNs is of importance, because they may pose serious risks to health if they accumulate within the body, such as has been seen with MSN-hydroxyapatite (MSNs/HAP) [87, 88].

Mesoporous silica microspheres, however, are only appropriate for non-biological applications, because microspheres are too large to pass easily through capillaries and to be taken up by cells. Microspheres also face the risk of being phagocytosed by macrophages and being eliminated by the immune system [84]. MSNs have mostly been focused upon addressing the above-mentioned issues.

Wang *et al.* showed the SR of DOX plus curcumin (CUR) using a core-shell structure nanocarrier based on polydopamine-MSN@zeolite imidazolate frameworks-8 (PDA-MSN@ZIF-8) with a microporous ZIF-8 shell and a mesoporous PDA-MSN core, designed to overcome the MDR of cancer cells [89]. In this nanocarrier, PDA, which functioned as a gatekeeper on the surface to block the pores of the MSNs, was extremely sensitive to pH variation triggered by its interaction with inorganic, organic, and hybrid materials [90, 91]. At the low pH of cancer cells, CUR was released in the cytosol of the cells and acted as a P-glycoprotein (P-gp) inhibitor to block the efflux of DOX, and facilitate the nuclear transport of DOX with a corresponding increase in the effectiveness of its antitumor activity. While PDA-MSN@ZIF-8 was reported to be highly biocompatible and non-cytotoxic, its combination with DOX, (and in particular with CUR + DOX) was highly cytotoxic, and hence more effective.

In another study conducted by Saiyin *et al.* polymeric micelles (PMs) were used for the SR of two antitumor drugs to overcome MDR, by preventing tumor cell autophagy thus making them more sensitive to DOX in the treatment of oral squamous cell carcinoma (OSCC) [92]. PMs obtained from the self-assembly of block copolymers are among the most useful nanocarriers for SR drug delivery application, due mainly to their small size (<200nm), high solubility, excellent biocompatibility, and versatile preparation methods [93–95]. Drugs can be linked to the PMs using pH-labile chemical linkers, including hydrazone, oxime, acetyl, and imine linkages [95]. The pH variations in the environment of tumor cells result in breaking the bonds in the linkers leading to SR of the drugs. When pH-sensitive PMs are exposed to relatively low pH values, the hydrophobicity of micellar hydrophobic segments is reduced, which causes the micelles to swell and release the drug or gene contents. In this

process, the PMs become protonated, and the amphipathic structure of the micelles is therefore degraded. In the study mentioned earlier, DOX was conjugated to hydrophilic and pH-sensitive hyperbranched polyacylhydrazone (HPAH) through an acylhydrazone linkage, while the autophagy inhibitor LY294002 (LY) was encapsulated in the core of the self-assembled HPAH-DOX micelles. Hydrazone is considered to be one of the most useful pH-labile chemical bonds and is widely used in conjugating anticancer drugs to PMs. On the other hand, solubility is of vital importance for the NPs used in drug delivery. HPAH has good solubility and low toxicity as a result of containing many end groups of acylhydrazone. In this study, the acylhydrazone linkages broke down under the acidic conditions of tumor tissues leading to drug release, while the LY was released faster than DOX, making the tumor cells more sensitive to DOX by inhibiting autophagy. It was concluded that the sequential co-delivery of a chemotherapy drug (DOX) and an autophagy inhibitor (LY) using pH-responsive polymer nanomicelles could enhance the efficacy of the drug, prevent the proliferation of cancer cells, and increase cell apoptosis (Fig. 3).

Chitosan polymers have been investigated as drug/gene delivery carriers over the last several years, due to their pH-sensitive properties, biodegradability, and biocompatibility [96]. These cationic polymers are found in nature, and despite having many amine groups and a positive charge, they are less immunogenic than other polymers. With a positive charge, chitosan has the ability to bind to negatively charged polymers, nucleic acids, mucus membranes, as well as epithelial cells, facilitating the delivery of high molecular weight and hydrophobic drugs[1]. The proper modification on the surface of chitosan NPs also makes them suitable for drug/gene delivery and multifunctional imaging [97]. However, the low solubility of these polymers in water above pH 6, has limited their use for encapsulating hydrophobic drugs, and requires the addition of acid to ensure the protonation of the amine groups, which limits its modification for the intended purposes and causes toxicity [98]. Therefore, the use of other derivatives of chitosan conjugated with hydrophilic groups such as glycol chitosan (GC) can solve this problem to some extent.

Yoon *et al.* developed two GC-based NPs (CNPs), one containing DOX and the other containing Bcl-2 siRNA (triggering cancer cell apoptosis), which overcomes drug resistance independent of MDR efflux pumps. The nanoplatform was composed of CNPs encapsulating DOX by its hydrophobic interaction with the cholanic acid moieties of GC polymers (DOX-CNPs), and CNPs encapsulating Bcl-2 siRNA through electrostatic interactions (siRNA-CNPs). Since the entire surface area of both types of CNPs was covered with GC polymers, the protonation of the amine groups of the GC polymers and their ionization resulted in the instability of the CNPs structure, thus co-releasing DOX and siRNA. The co-delivery of DOX and siRNA together could increase the efficiency of cancer treatment because DOX can act as an apoptosis-inducing anticancer drug, while Bcl-2 siRNA suppressed the Bcl-2 as an apoptosis-inhibitor. The most distinctive features of this nanoplatform were its significant efficacy, reduced toxicity, high drug loading capacity, significant accumulation at tumor sites, and long-term tumor growth inhibition, which altogether made it promising for drug-resistant cancer therapy [99].

The therapeutic efficacy of anticancer drugs, especially in the case of MDR cells, increases if these drugs can overcome biological barriers by, for instance allowing endosomal escape,

and reach the cell nucleus. Hence, NPs face serious challenges in nuclear drug delivery due to their short circulation time and degradation in endosomes. To address these particular issues, drug carriers have been designed based on polymers. Polymers not only can control drug release, but also protect the drug against environmental humidity, and prevent it from being destroyed during its passage through the digestive tract and cellular endosomes [100].

In a recent study, novel nanocarriers were synthesized using a pH-sensitive nanosystem composed of an interior core of smaller NPs (CS-polyacrylicacid NPs) (CS/PAA NPs) with a confined size, externally surrounded by a larger shell of NPs (Vitamin E/ tocopheryl-PEG modified PLGA (TPGS/PLGA NPs). This system which was abbreviated as S@LNPs allowed endosomal escape and passing through nuclear pores, and the smaller NPs were taken up by MDR cells. When exposed to the acidic environment of the endosomes or lysosomes, the larger outer NPs were degraded releasing the smaller NPs, which in turn, when exposed to the alkaline environment of the nucleus were degraded, and therefore released their cargo of etoposide (VP-16) to inhibit the synthesis of DNA and topoisomerase II. It was reported that the cytotoxicity of VP-16 toward A549/DDP cells showed a nearly two-fold increase when loaded into CS/PAA NPs, and a nearly three-fold increase when loaded into CS/PAA@TPGS/PLGA compared to the free drug. The encapsulation efficiencies (EF) of CS/PAA/VP-16 NPs and CS/PAA/VP-16@TPGAS/PLGA were respectively 88.9% and 91.7%, while their loading efficiencies (LE) were 4.21% and 1.35%, showing the potential of these NPs for drug delivery applications [100].

Core-shell nanocarriers have many benefits, such as facilitating the simultaneous encapsulation of both hydrophobic and hydrophilic drugs/genes for SR-based nanosystems by loading therapeutic agents into the core and shell regions of the carriers [101, 102].

One of the obstacles standing in the way of treating some diseases after drugs are absorbed from the gastrointestinal tract is that the plasma concentration suddenly increases, thus necessitating controlling the dosage to maintain this concentration at the desired level for the next few days. Core-shell carriers could be a good choice for designing a drug release system that is able to maintain the drug concentration at a controlled level for a specific length of time (whether hours or days) using a combination of different release modes. Yang *et al.* developed pH-sensitive core-shell chitosan microcapsules that could carry out SR using two modes of burst release and sustained-release in order to deliver two anti-inflammatory drugs (lipophilic CUR and hydrophilic catechin) for the treatment of acute gastritis. As shown in Fig. 4a1, the structure of these microcapsules includes a pH-sensitive terephthalaldehyde cross-linked chitosan hydrogel shell and an oily core containing lipophilic and hydrophilic drugs. In the burst release step, the chitosan shells of the microcapsules decomposed in the acidic medium of the stomach, and released both the drug-loaded PLGA cores and the drug molecules (56.2% of CUR and 59.6% of catechin) (Fig. 4a2–a3), followed by the sustained-release step, in which the PLGA cores were degraded over two days providing a sustained concentration of the drugs (19.3% of CUR and 32.3% of catechin) (Fig. 4b1–b3) [20].

Dendrimers are a group of macromolecules with structurally symmetric branches originating from a central nucleus, with unique physical and chemical properties due to their well-

defined 3D structure [103, 104]. Dendrimers are widely used for biomedical and industrial applications, especially in DDSs, due to their uniform size, water solubility along with very narrow polydispersity (Mw/Mn~1.00–1.05), homogeneous and monodisperse structure, as well as their potential for possessing large numbers of branches according to their generation number [105, 106]. The central core, internal space, and the surface groups of dendrimers can all be used for loading drugs in delivery systems, either by electrostatic interaction or encapsulation of drugs into cavities [107]. Although these characteristics of dendrimers make them suitable for drug/gene delivery, these NPs suffer from disadvantages, such as a complex synthesis process and limitated incorporation of drugs into the cavities. Today, some of these problems have been addressed with modifications and changes in the structure of the dendrimers, such as altering the number of carboxyl and amine functional groups to increase the loading capacity [108]. The hemolytic toxicity of dendrimers has also been reported when used for DDs applications. This toxicity is thought to depend on the cationic charges on their surface groups. Nonetheless, a number of dendrimer-based systems have recently been developed to reduce this toxicity and make them more suitable for DGDSs. These systems often rely on the use of surface PEGylation, to reduce the hemotoxicity and provide additional advantageous properties [109].

Acid-labile bonds such as hydrazone bonds, boronate ester bonds, and acid-activatable ligands, including pH (low) insertion peptides (pHLIP) can be formed between drugs and dendrimers, resulting in pH-sensitivity [110]. Acton *et al.* studied the first and second generation of Janus PEG-based dendrimers for delivery of two different “model drugs”, benzyl alcohol (BA) attached by carbonate linkage and 3-phenylpropionic acid (PPA) attached by ester linkage. They, therefore, compared four different dendrimers (BA)4-G2-G2-(PPA)4, as well as (BA)2-G1-G1-(PPA)2, (BA)4-G2-G1-(PPA)2, and (BA)2-G1-G2-(PPA)4 and looked at the stability under different physiological conditions including pH 7.4, pH 5, and human plasma at 37 °C. Amide and carbamate linkages showed higher stability than ester and carbonate linkages. The branched linkages between tertiary amine groups and PEG can provide better stability to ester and carbonate linkages allowing them to function in SR due to their faster hydrolysis in plasma. The drug release in the plasma at 37 °C, at pH 7.4, and at pH 5 showed an increasing trend, and BA was released faster than PPA due to the higher degradability of carbonate linkage compared to ester, leading to the SR of the drugs so that one drug was released before the other. By measuring the rate of hemolysis and the cytotoxicity caused by Janus PEG-based dendrimers, it was found that they were nontoxic and biocompatible [46].

In the field of tumor nanomedicine, the dissimilarity and complexity of tumors has been considered as a challenge that necessitates the development of new carriers and targeting agents for each cancer treatment. However, the mechanism of accumulation of nanocarriers in solid tumors coulkd be governed by a conserved pathway. The enhanced permeability and retention (EPR) effect could be considered to be the underlying concept behind this phenomenon, which is mostly encountered in tumors with a leaky vasculature and poor lymphatic drainage. Also, other factors including particle size, morphology and structure, surface properties and active targeting strategies, could be considered for engineering nanoplatforms for solid tumor-therapy [111]. In this regard, techniques based on PEGylation are a promising strategic option for the delivery of siRNA with improved efficiency. The

mechanism of this delivery system is based on cleavage of PEG chains at the acidic pH in the tumor environment, which leads to improved cellular uptake as well as increasing the blood circulation time. The use of PEG will prolong the blood circulation time, but it could also decrease the accumulation in the tumor cells. Therefore, PEG functional groups connected by pH-sensitive linkers like poly(2-(hexamethyleneimino)ethyl methacrylate) (PHMEMA), could be degraded once they have reached the tumor to release the cargo in a sequential manner [112].

A multistage pH-responsive nanoplatform based on pH-responsive PEG-*b*–PHMEMA was also used for siRNA delivery with additional targeting based on bromodomain 4 (BRD4). This was studied as a targeted agent for castration-resistant metastatic prostate cancer. BRD4 could interact with the androgen receptor in prostate cancer, and disrupt its proliferation. *In vivo* experiments showed that this multistage carrier had a long blood circulation time, enhanced accumulation at the tumor site, showed BRD4 targeting, allowed siRNA delivery to the cytosol, and resulted in effective gene silencing [112].

3.2. Redox reactions

Redox-sensitive DGDSs have been designed based on the difference that exists between the redox potential of the normal cells in the body and tumor cells. One of the most common redox pairs is the reduced glutathione (GSH) and glutathione disulfide (GSSG) pair. In addition to their lower pH, tumor tissues also have a lower level of oxygen compared to the normal tissues of the body, as well as a higher concentration of GSH that can reach 4 times that of normal tissues, enabling the development of redox-sensitive SR-capable DGDSs. The function of redox-sensitive systems is similar to GSH, mainly causing the breakdown of disulfide bonds as well as ditelluride bonds by the action of a reducing agent. Reducing agents such as GSH cause cysteine thiol groups to lose their protons, and GS-GS bonds are subsequently formed. Drug release occurs because disulfide bonds are broken down into sulfhydryl groups, which results in the degradation of the carrier and the release of the cargos [113–115].

Hydrogels can be classified according to their origin, including natural (alginate, chitosan, collagen, fibrin, and gelatin) and synthetic [116, 117]. For many years, natural polymers such as chitosan and alginate have been used to develop hydrogels [118]. Hydrogels can also be synthesized from a broad range of synthetic monomers including hydroxyethyl methacrylate (HEMA), methoxyethyl methacrylate (MEMA), ethoxydiethoxyethyl methacrylate (MDEEMA), ethylene glycol (EG), PEG acrylate (PEGA), acrylic acid (AA), and vinyl acetate (Vac) [119]. Due to their special structure consisting of a porous polymeric network, hydrogels are attractive choices for SR-based DDSs, because they allow both hydrophilic and hydrophobic drugs to be loaded into their pores [120, 121]. Drug loading can be performed by dissolving or encapsulating drugs in the hydrogel network, and genes can be electrostatically bound to the charged hydrogel network. The high absorption rates of hydrogel water speed up the drug release, especially hydrophobics from gels, faster [122–125].

Hybrid systems based on a combination of NPs and hydrogels or lipid polymer hybrid NPs have used for SR of drugs or genes. These systems offer many advantages such as

maintaining structural integrity and enabling the SR of multiple drugs, enhancing their therapeutic effects while reducing their side effects by lowering the overall dose of a drug [126, 127]. In one recent study, a hybrid system was designed with a combination of hydrogel and liposomal NPs for the co-delivery of DOX and cytochrome c. A hierarchical-nanogel structure allowed the encapsulation of several therapeutic molecules followed by SR. The system was composed of glutathione-sensitive-liposome-cross-linked hybrid hydrogels based on a reversible micelle-formation between the arylthiol-functionalized 4-arm PEG and a maleimide-functionalized liposome [128]. GSH disrupted the structure of the hydrogels due to the presence of glutathione-sensitive thioether succinimide linkages, which facilitated the SR of two therapeutic agents. One of the advantages of this hybrid system was to encapsulate hydrophilic drugs in the gel, providing a rapid release, and to encapsulate hydrophobic drugs in the NPs, allowing either simultaneous release, or SR with different release profiles. Another advantage of this hybrid system was its relatively simple synthetic procedure.

In certain tumors like hepatocellular carcinoma, p53 which acts as a tumor suppressor gene is mutated and dysfunctional. A redox-responsive lipid polymer hybrid nanoparticle was utilized to encapsulate *p53*- messenger RNA (mRNA) and deliver it to the cytoplasm. It was found that this complex system could successfully inhibit the growth of *p53*-null hepatocellular carcinoma and non-small cell lung carcinoma (NSCLC) cells through cell cycle arrest and inducing apoptosis *in vitro* and *in vivo* [129].

3.3. Enzyme reactions

Enzyme-responsive DGDSs are another useful type of SR delivery system based on the advantageous properties of enzymes, including their high expression levels, up-regulation under different pathological conditions (inflammation, cancer, and infections), activity at isoelectric pH, specific catalytic reactions, and their physiological functions within the human body [113]. Various types of enzymes have been employed in enzyme-responsive DGDSs, such as proteases, phospholipases, elastase, hyaluronidase oxidoreductase, hyaluronidase, and MMPs [4].

The mechanism of enzyme-responsive DGDS can be either physical or chemical release. In the case of the physical mechanism, therapeutic agents are released based on enzyme-catalyzed changes occurring on the surface of the NPs. In this process, enzymatic reactions do not degrade the actual structure of the NPs, but alter the functionalities on the surface, thus releasing the drugs. While in the case of the chemical mechanism, the nanocarriers are synthesized in such a way that their actual structure is sensitive to specific enzymes, and the carriers that encapsulate the drugs are degraded [130]. A wide range of enzyme-responsive nanomaterials has so far been developed, including dendrimers and liposomes [131]. In the synthesis of dendrimers, enzyme-labile bonds can be used in the branches. These could be peptide linkers such as Gly-Phe-Leu-Gly (GFLG), azo-containing linkers that can be broken by azoreductase enzyme, and PVGLIG linkers that can be hydrolyzed by MMPs are examples [110].

Li *et al.* designed an amphiphilic dendrimer engineered nanocarrier system (ADENS) using the amphiphilic dendrimer of G0-C14. The system encapsulated siRNA within its

hydrophilic outer layer and paclitaxel (PTX) within its hydrophobic inner core (Fig. 5a). In this study, MMP2/9 played an important role in the hydrolysis of PVGLIG linker thus producing the ADENS-cell-penetrating peptides (ADENS-CPP), which improve the intracellular penetration of the nanocarriers, encouraging uptake into endosomes, allowing endosomal escape, and finally to enter the cytosol. Anti-vascular endothelial growth factor siRNA (anti-VEGF siRNA) was loaded into the dendrimer. This siRNA was used to silence VEGF gene expression to produce an anti-angiogenesis effect within the cytosol. The tumor microenvironment-sensitive polypeptides (TMSP)-ADENS caused the siRNA to be released from endosomes and then to the cytosol, and the PTX provided additional cytotoxicity toward the tumor cells (Fig. 5b). The TMSP-ADENSs alone (no loaded cargos) were found to be safe and biocompatible and could be promising vehicles for drug delivery. The simultaneous delivery of PTX and siRNA reduced tumor growth as well as the toxicity of siRNAs and lowered the chemotherapy dose[132].

Hyaluronidase (HAase), is an enzyme that is over-expressed in tumor cells, encouraging metastasis, angiogenesis, and cellular invasion by degrading the extracellular matrix [133, 134]. Jiang *et al.* took advantage of this property to design a core-shell carrier composed of a liposomal core and a cross-linked gel shell (Gelipo) for the SR and site-specific delivery of DOX (loaded in the liposomal core) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) encapsulated in the shell. Here, the outer shell was made up of cross-linked hyaluronic acid and was degraded by the HAase enzyme and causing TRAIL to be released which in turn activated the caspase 3 signaling pathway and led to cell death. The subsequent release of the (CPP, R8H3)-modified liposomal core improved the tumor cell uptake of the liposome, allowing endo-lysosomal escape, releasing DOX to the nucleus, and inducing cell apoptosis [131].

Enzyme-sensitive drug release systems can also be used for the effective delivery of growth factors and proteins to accelerate tissue regeneration and wound healing. Zhu *et al.* designed a special delivery platform that was sensitive to proteolysis by tissue-proteolytic enzymes, based on enantiomeric protein nanocapsules homogeneously dispersed in an injectable hydrogel. These were intended for the SR of VEGF and platelet-derived growth factor (PDGF) to help tissue repair and wound healing in mice. The nanocapsules consisted of plasmin-sensitive L and D enantiomeric cross-linkers formed from plasmin-sensitive monomers with neutral, positive, or negative charges on the outer shells. It was observed that the released growth factors could trigger the formation of granulation tissue and increase blood vessel density with more pericyte coverage. After injecting the hydrogel into a scar, the nanocapsules became uniformly dispersed and were exposed to the proteolytic trypsin enzyme present within the scar. The trypsin degraded the cross-linkers at different rates (L faster and D slower), leading to the release of VEGF in the first three day period, and release of PDGF in the second three day period [135].

3.4. Light irradiation

Light can function as a non-invasive external stimulus factor that has been extensively used in release systems because it is easy to produce, non-invasive, and highly controllable. A wide range of optical wavelengths has so far been used for triggering optical responses, such

as ultraviolet (UV) (100–400 nm), visible light (400–750 nm), and near-infrared (NIR) (650–900 nm) [136, 137]. UV light is to able to deliver the transfer of higher energy per photon so that more efficient photochemical reactions can be carried out, but its penetration depth into tissue is limited, and it may cause serious damage [136]. NIR light, on the other hand, can penetrate deeply into tissues in the *in vivo* environment and is safer for the body, but its lower energy per photon is not of much interest for photochemistry [138]. Visible light can be used to penetrate shallow tissues such as the skin [139]. Some of the most important mechanisms of light-triggered drug/gene release include isomerization, cross-linking, reduction, and oxidation. In addition, photon upconversion NPs (UCNPs) and two photon absorption are non-linear optical processes. In recent years, many therapeutic approaches have also been developed based on light-sensitive NPs, such as photodynamic therapy (PDT), photothermal therapy (PTT), radiodynamic therapy, and light-triggered drug delivery systems [136]. Different types of light-sensitive nanocarriers have been designed on the basis of irreversible photocleavage reactions, or reversible photoisomerization photochromic materials such as coumarin (Cou) [140], perylen-3-ylmethanol [141], o-nitrobenzyl [142], and p-hydroxyphenacyl [143]. When exposed to some wavelengths of light such as UV, the chemical structure of photochromic materials undergoes changes, thus bringing about a shift in the absorption coefficient of the material.

In a study conducted by Wu *et al.*, MSNs were functionalized with two different photochemical materials, Cou and o-nitrobenzyl. These photo-responsive mesoporous NPs (PMSN) were used for the co-delivery of short-hairpin RNAs against P-glycoprotein, plus DOX as a cytotoxic drug (shRNA P-gp/DOX) in order to increase the effectiveness of the treatment and to overcome the MDR in HepG2/ADR human liver cancer cells. As shown in Fig. 6b, the surface of the PMSNs was functionalized with Cou-poly[(dimethylamino)-ethylmethacrylate] (Cou-PDMAEMA), producing MSN-Cou-PDMAEMA. These PMSNs were able to compress nucleic acid strands to form polyplexes, which facilitated cell uptake and the endosomal escape of genes. DOX and shRNA were sequentially released inside the MDR tumor cells using the PMSNs. The shRNA was released after the Cou linker was photolyzed by 405 nm irradiation and the DOX was released after the hexadecyl-o-nitrobenzyl derivative-caged DOX (DOC) in the inner pores of the PMSNs was photolyzed by 365 nm irradiation. The results showed that PMSNs were promising for the SR of shRNA and DOX in both *in vivo* and *in vitro* studies by inhibiting P-gp activity in MDR cancer cells and improving the effects of DOX. Moreover, the toxicity of the PMSNs against HepG2/ADR cells was compared with polyethylenimine (PEI, another non-viral gene carrier) using the CCK8 method. The toxicity of PMSNs was lower than that of PEI, which highlights their potential as a gene carrier with controlled release [27].

The effectiveness of cancer treatment can be increased by combining PDT with chemotherapy. PDT uses a photosensitizer (PS) and a light source in order to produce reactive oxygen species that kill cancer cells. In a recent study, Fan *et al.* prepared large and small self-destructing NPs with a size of 200 nm and 50 nm, respectively, and encapsulated the PS, methylene blue in the core of $NPs_{large\ \&\ small}$ for PDT, and gemcitabine hydrochloride (GM.HCl) as a cytotoxic drug. Under light irradiation, the GM was rapidly released from the NP_{large} simply because of their greater volume, leading to improved chemotherapy, followed by the SR of the methylene blue with two release peaks. This SR

pattern overcame the P-gp-mediated efflux, resulting in the overall enhancement of chemo-photodynamic therapy. There was a significant decrease in the tumor size due to the simultaneous effects of $NP_{large\ \&\ small}$ with light irradiation without any side effects or inflammation [144].

In another study, the same group investigated the SR of three different drugs including methylene blue, GM, and docetaxel (DTX) from three different types of NPs. These were: (a) large (average diameter of 200 nm) and thin; (b) large and thick; (c) small with an average diameter of 50 nm. Similar to the previous study, the encapsulation of methylene blue into the core of each of the three NPs, plus electrostatic adsorption and pore adsorption of GM.HCl to $NP_{large\ \&\ thin}$ and the pore adsorption of DTX to $NP_{large\ \&\ thick}$ was performed. According to the results, the release of methylene blue indicated three peaks, maximizing the PDT effect; GM.HCl was released faster than DTX from the outer layer of $NP_{large\ \&\ thin}$ and $NP_{large\ \&\ thick}$ due to the charge effect in the release medium, while the release of GM and DTX with a 12-h time lag enhanced their chemotherapeutic efficacy [145].

3.5. Temperature change

Temperature is one of the most effective and suitable stimulus factors (that can be either internal or external) to provide SR in DDSs. Tumors and inflammatory cells have inherently higher temperatures than normal cells and tissues, due to their elevated metabolic rates, however, these differences are usually slight. Applying an external temperature source can be used to activated a thermosensitive nanocarrier to increase the drug/gene release rate at targeted sites as a result of their rapid response to thermal change [26]. Many polymers have a critical temperature at which they undergo a phase change. There are two types of polymers that display critical temperatures. Polymers that have an upper critical solution temperature (UCST), above which their components are miscible in all proportions; examples include acrylamide (Aam) and acrylic acid (AA) [146]. Other polymers have a lower critical solution temperature (LCST) below which they have a single phase, such as poly(N,N-diethylacrylamide (PDEAAm)) which is often used for delivery systems [26, 147].

Some polymers including PLGA can be suitable carriers for the SR of genes and drugs due to their excellent degradation properties, long circulation times, site-specific drug delivery, and gradual release. A number of strategies have so far been used to reduce the toxicity of NPs, such as the use of polymeric NPs like PLGA, because of the fact that the shell around the NPs can reduce their interaction with normal cells and tissues of the body, and thereby reduce their toxicity [148]. However, the low drug loading, especially of small hydrophilic drugs, is a barrier to the widespread use of stable formulations PLGA for clinical use. Synthesis of PLGA microspheres using a double emulsion method in order to increase the drug loading, or other techniques such as coaxial electro-spraying could solve this problem [149, 150].

In a study carried out by Zheng *et al.*, a thermosensitive hydrogel–microsphere (Gel-MP) system with high loading capacity and a good biodegradability rate was designed based on poly(L-alanine-*co*-L-phenylalanine)-*b*–PEG*co*-poly(L-alanine-*co*-L-phenylalanine) (PLAFb-PEG-b-PLAF) and PLGA for the *in situ* SR of DTX plus combretastatin A-4(CA4)

for osteosarcoma treatment. The PLGA MP synthesis by the double emulsion technique gave an average size of approximately 2.94 μm, and DTX could be efficiently loaded into the MPs with 98.8 wt.% of drug loading efficiency (DLE). The thermosensitive Gel/CA4-MP/DTX hydrogel was stirred at 4°C and injected into the body, and then the drugs were released at body temperature. It was observed that CA4 was initially released from the hydrogels leading to the rapid destruction of tumor blood vessels, causing a reduction in nutrient delivery to the tumor, leading to improved DTX penetration into the tumor and better cytotoxic effects. The results of *in vivo* studies in mice indicated that the injection of Gel/CA4/MP-DTX enabled a sequential and site-specific drug release, with reduced drug toxicity and lower damage to adjacent normal cells, and inhibited the growth of osteosarcoma tumors in mice. *Ex vivo* histological analysis of sections taken from various mouse organs showed the non-toxicity of Gel-MP, and the reduced side effects of the drugs [150].

3.6. Magnetic fields

Magnetic fields can be used as an external triggering factor in drug delivery systems. It has been claimed that magnetic fields physically interact with the body, more strongly than any other forces [26]. DGDSs have been developed based on iron oxide NPs (IONP) with a number of favorable properties, including superparamagnetic and supersaturation properties, originating from the intrinsic properties of these NPs [26, 151]. The stability of MNPs can be increased by means of different surface coatings, such as organic materials (polymers), inorganic materials (transition metal-based and mixed metal oxides), which also prevent the excessive interaction of MNPs with cells, thus increasing their biocompatibility. Due to their stronger magnetic properties and higher biocompatibility, super-MNPs have been extensively employed to create magnetic carriers of drugs or genes for magnetic hyperthermia and for SR [152]. Magnetoliposomes have recently attracted considerable interest as biosensors and for imaging, because of their amphiphilic properties. Magnetoliposomes can facilitate the SR of therapeutic agents due to the magnetic behavior of the superparamagnetic iron oxide NPs (SPION), their biocompatibility, and their ability to respond to externally applied magnetic fields to control drug release [153].

Salvatore *et al.* prepared multifunctional magnetoliposomes for an SR-based delivery system, composed of 1, 2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) multilamellar polydisperse vesicles, double-stranded DNA (dsDNA), and compared hydrophobic and hydrophilic SPIONs each of 5 nm in diameter. Two different types of MNPs were used: hydrophobic Fe_3O_4 NPs and hydrophilic Fe_3O_4 NPs both enwrapped in gold shells (Au@Fe_3O_4 NPs). The synthesized Au@Fe_3O_4 NPs were functionalized with zipper ON (therapeutic dsDNA hybridized with a zipper) and cholesteryl-ON. When this system was loaded with carboxyfluorescein (a model hydrophilic fluorescent drug) and zipper ON, carboxyfluorescein was first released after exposure to a 3.22 kHz alternating magnetic field (AMF) over a short period of time, while after longer exposure to a 6.22 kHz AMF, zipper ON was released (Fig. 7). The mechanism was to increase the permeability of the liposomes by AMF heating, leading to releasing of the cargo from inside the liposomes by application of lower frequency AMF. A higher frequency AMF was then used to produce enough heat to disrupt the dsDNA melting point leading to the release of zipper ON. There was no size

change of the SPIONs during in this process. This technique enables controlled SR for delivering therapeutic agents by applying an external magnetic field. Magnetoliposomes, on the other hand, facilitate SR and increase the effectiveness of drugs even at low magnetic field frequencies [154].

3.7. Sequential release systems based on dual/multi stimuli

Dual/multi-responsive DGDSs are created by combining NPs that are responsive to two or more different internal or external stimuli, thus increasing the accuracy and efficiency of CR. The combinations of two or more stimuli-responsive systems can compensate for the shortcomings of single stimulus-responsive systems, leading to better biodistribution and pharmacokinetics of drugs, and their better absorption, as well as simultaneous release [4, 155]. However, the multi stimuli are more promising than two stimuli in delivery systems, and it widely increases agent release and accumulation in the targeted sites, thereby improving the therapeutic effects of the drug/gene without inducing side effects.

There are several different types of these dual responsive systems including, light/pH-responsive [156], pH/temperature-responsive [157], magnetic field/pH-responsive [158, 159], pH/redox-responsive, light/temperature [160], magnetic field/temperature [161], etc.

3.7.1. Dual light/pH-responsive systems—Dual light/pH-responsive systems are one of the most important groups of dual responsive systems for the SR of drugs/genes. Light can act as an external stimulus in DGDSs, yet in addition to its advantages, it also has limitations; for instance, UV light is only applicable to superficial tissues such as the skin, while NIR photons have too low an energy to carry out photochemistry. The heat generated by an external light source may carry a risk of causing damage to the surface of the tissue instead of penetrating into the deep tissue where it is actually required. Hence, the application of an internal stimulus in combination with an external light stimulus could increase the efficiency of dual/multi-responsive DGDSs [136].

AuNPs are widely utilized in biological applications due to their biocompatibility, low toxicity, and outstanding optical properties. These applications include biosensors and DGDSs, among others. Their simple synthesis process provides a uniform particle distribution, and the possibility of functionalizing the surface with different molecules is an additional advantage [162–166]. Plasmonic metal NPs possessing localized surface plasmon resonance (LSPR), such as gold nanostructures, have been shown to be excellent tools for the production of cancer hyperthermia by absorbing light in the NIR region, as well as rapidly producing substantial heat within seconds or less [11]. Gold nanorods (AuNRs) are one sub-type of AuNPs, often used in PTT-based delivery systems [167].

In one recent study, Song *et al.* constructed a dual light/pH-responsive delivery system based on reduced graphene oxide (rGO)-loaded ultra-small plasmonic gold nanorod vesicles (rGO-AuNRVe) for the SR of DOX. Small AuNRs were coated with PEG and PLGA, forming AuNR@PEG/PLGA, while DOX and DOX loaded rGOs were loaded into the hybrid rGO-AuNRVes, to provide SR for cancerous tissues. NIR induced DOX release from the vesicles by increasing the temperature, while the intracellular acidic environment-induced DOX release from the surface of the rGOs (Fig. 8). The intravenous injection of these hybrid

vesicles into the tumor-bearing mice caused the vesicles to become aggregated within the tumor tissues due to their small size (65 nm), so that photoacoustic (PA) signals could be detected at the aggregation site within the tumor area. The results showed the efficacy of rGO-AuNRVe-DOX in tumor treatment in an *in vivo* environment using PTT and augmented by the CR of chemotherapeutic agents. In the absence of laser irradiation, no DOX release was observed, and the rGO-AuNRVes showed no toxicity; however, upon being exposed to laser irradiation, rGO-AuNRVes exhibited cytotoxicity, suggesting the importance of integrating PTT with chemotherapy [168].

Zhang *et al.* constructed a dual light/pH-responsive system by synthesizing caged–spherical amphiphilic poly(3-caprolactone)-gold nanocages coated with ferric hydroxide-poly(acrylic acid) as Janus NPs (PCL-AuNC/Fe(OH)3-PAA JNPs) for the sequential delivery of two drugs; hydrophilic DOX and hydrophobic DTX. This novel multifunctional system showed advantages over conventional single-drug delivery systems, enabling the transfer of hydrophilic and hydrophobic drugs loaded in two domains, each of which could be released by an independent stimulus while providing the CT/MR imaging capability in *in vivo* experiments. Other features of this example are described in section 2.1 under the category of Janus NPs [19].

Application of an external alternating magnetic field (AMF) to IONPs can generate heat and cause tissue hyperthermia, thus increasing the temperature of the tumor above 42°C, and increasing the sensitivity to chemotherapy drugs. [169]. For instance, Benyettou *et al.* used CB[7]-modified IONPs for the SR of two drugs, zoledronic acid (Zol) and DOX, to MCF-7 breast cancer cells. Zol increases bone density and has anti-osteoporosis properties. This drug was bound to the NPs by ionic bonds formed between Fe^{2+} and three phosphonate oxygen atoms, leading to the formation of a Z-NP complex. Next CB[7] was bound to Zol via an imidazole group at pH 7.4 and room temperature, producing the CZ-NP complex. DOX was then attached to the complex, and the final DCZ-NPs complex was stable at room temperature and at pH 7. It was found that the application of the DCZ-NPs complex to MCS-F cells followed by their exposure to AMF caused the sudden release of DOX due to the rise in temperature to 42 °C, and due to the low pH (5.4). Zol then began to be gradually released at low pH, because the bonds between Zol and NPs were weakened under acidic conditions, and just two phosphonate oxygen atoms remained to carry out binding. The cytotoxicity measurements gave the IC50 values of free Zol, Z-NPs, CZ-NPs, DCZ-NPs, and the latter showed the highest cytotoxicity compared to the other compounds [170].

3.7.2. Dual light/redox-responsive systems—DGDSs have been designed based on light/GSH dual stimuli-responsive release to facilitate the release of drugs/genes directly into the cytoplasm of tumor cells, thus reducing the side effects of the drugs/genes on normal cells. Wu *et al.* developed GSH/light dual responsive polymeric prodrug NPs, using the self-assembly of disulfide-containing alkyl-modified polyethylenimine (C16-S-S-PEI) and PLGA. These were designed for SR of siRNA against the P-gp gene and DOX. Light destroyed the photochromic groups of the nitrobenzyl bonds, leading to the release of the caged DOX. DOX was conjugated to the hexadecyl chains through a linker of 5-hydroxy-2-nitrobenzyl alcohol, which prevented the early release of the drug. DOX was loaded into the PLGA core, and the P-gp siRNA was adsorbed onto the cationic polymeric shells. After the

uptake of NPs/DOC/siRNA into MCF/ADR cells, the reduction of the disulfide bonds by cytoplasmic GSH caused siRNA to be released and in the following leads to suppression of P-gp expression, which by itself caused the release of DOX by the irradiation of light. The cytotoxicity of DOX was increased in both the *in vivo* and *in vitro* environments as a result of employing P-gp siRNA in MDR cancer models [29].

3.7.3. Dual pH/redox-responsive systems—The rate of drug release can be increased by removal of the gatekeepers or caps from the pores of the nanocarriers. This release can be based on dual redox/pH-responsive systems with synergistic activity uder high redox and low pH conditions. Gatekeepers play a pivotal role in SR by controlling the mesopores of porous nanocarriers such as MSNs and mesoporous carbon NPs (MCNs). There are different types of gatekeepers, including polymers, biomacromolecules, and iONPs [171]. Their role is to prevent the premature leakage of drugs or genes loaded inside the pores, before the NPs reach their target site, while their release can be triggered by various stimuli such as temperature, pH, light etc. whether inherent to the tumor or focused onto the tumor from the outside [171, 172].

Palanikumar *et al.* synthesized polymeric-gatekeeper hollow mesoporous silica NPs (HMSNs), programmed to open the gates in an acidic environment, or in the presence of the cytosolic GSH levels found in tumor cells. In this study, the cationic gatekeepers presented positive charges, while the pyridine disulfide (PDS), 2-(diisopropylamino) ethyl methacrylate (DPA), with PEG bearing negative charges, were adsorbed onto the surface of HMSNs. The acidic environment of tumors led to the protonation of DPA, generating positive charges on the HMSNs and increasing cellular absorption. Under the even more acidic conditions of endosomes, the polymeric gatekeepers became swollen, followed by the release of a hydrophilic drug verapamil hydrochloride which inhibited the P-gp efflux pump. After being subsequently exposed to cytosolic GSH, the gatekeepers were totally destroyed due to the breakdown of the disulfide bonds, thus releasing the hydrophobic DOX and killing the cancer cells. An IC50 value of 1 μg/ml was reported here, indicating the higher cytotoxicity of DOX after verapamil had inhibited the P-gp-mediated efflux. [55].

In another study, Zhang *et al.* prepared polyacrylic acid-ss-mesoporous carbon NPs (PAA-ss-MCN) with an MCN core and pH-sensitive surface coatings for the SR of DOX. Here, the PAA acted as a pH-sensitive gatekeeper layer, which also contained carboxyl groups to increase the loading capacity of the drug because of increasing the electrostatic interactions. Glutathione-sensitive disulfide bonds were used as linkers to attach the DOX to the MCNs. The use of pH-sensitive polymeric coatings, and redox, all integrated into the one system to increase the redox effect. Once the NPs were exposed to the acidic environment of tumor cells, the PAA gating layer began to gradually release the drug, while in contact with GSH, the disulfide bonds became completely degraded, which facilitated the total release of DOX. According to the results of *in vitro* cytotoxicity tests, these NPs demonstrated acceptable compatibility, and drug-loaded DOX@PAA-ss-MCNs showed high toxicity [173].

Another pH/redox-sensitive system was devised based on magnetic supraparticles (MSP) for the SR of taxol (TXL) and DOX. One of the differences between this system and the delivery system that has been discussed above is that a different core-shell structure was

used with two different levels of sensitivity for two drugs. In this study, the MSP core was sensitive to acid, while the poly(methylacrylic acid-co-N,N-bis(acryloyl)cystamine) (P(MAA-Cy)) shell was sensitive to redox conditions, and the drugs were separately loaded into these regions. The resulting biodegradable MSP-TXL@P(MAA-Cy)-DOX system enabled the encapsulation of different molecules into different domains, thus releasing them sequentially into different regions of the cell. The cross-linked polymer shell was disrupted in the presence of 10 mM GSH, and subsequent protonation of the carboxylic acid groups led to the release of DOX. On the other hand, when the MSP core was exposed to the acidic pH of endosomes, the PTX drug was released. It was concluded that MSP-TXL@P(MAA-Cy)-DOX improved the killing effects against cancer cells with low cytotoxicity against normal cells suggesting its suitability for drug delivery applications. The MSPs also made it possible to monitor the destruction of the core releasing iron using an Fe^{3+}-selective fluorescent probe [174].

As mentioned earlier, the introduction of sequential drug/gene release systems has opened the door to the development of multidrug sequential delivery systems (*e.g.,* three drugs/genes), which could play an important role in the enhancement of anticancer drug efficacy by enabling the step-by-step release of three different therapeutic agents. In a study conducted by Fan *et al.*, SiO_2@AuNPs were employed for the SR of two drugs, 10-hydroxycamptothecin (HCPT) and DOX, plus one additional gene, Bcl-2 siRNA in colo-205 cells. During the synthesis of self-decomposing SiO_2 NPs, HCPT was loaded inside these modified NPs with the aid of 3-aminopropyltriethoxysilane (APTS), followed by the absorption of DOX onto the surface of the NPs; therefore, HCPT was located in the core and DOX was located on the surface of the NPs. The drug-loaded SiO_2 NPs were then coated with Au-PEG-mAb.198.3/siRNA NPs by means of electrostatic interaction. Thiolated-siRNA was bound to the gold via sulfide bonds and released from the NPs due to exchange with GSH. The two drugs (HCPT and DOX) were released under the acidic conditions of endosomes. The difference in T_{max} between these two anticancer drugs allowed a step-by-step release over an 8–12-hour period (rather than a simultaneous burst release of the drugs) thereby increasing their anticancer effect. This delivery system could reduce the expression of drug resistance genes prior to the drug release, and thus improve the cytotoxic effect and inhibiting tumor growth [30].

3.7.4. Other dual-responsive systems—In one recent study, a pH/cytochrome c (Cyt c)-dual responsive multi-organelle-targeted system was created based on dual dendrigrafted poly-L-lysines (DGL)-liposomal NPs (DGLipo NPs) composed of pH-sensitive liposomal shells and DGL cores for the combination therapy of resistant tumor cells using the SR of two anticancer drugs, DOX and the cyclopeptide RA-V. RA-V was loaded into the liposomal shells, while DOX was inserted into the DNA duplex of an aptamer that recognized Cyt c and encapsulated within the DGL core. The resulting DGLipo NPs were then modified by conjugating the integrin-binding peptide c(RGDfK) as well as the mitochondrial-penetrating peptide (MPP). The release of RA-V occurred at pH 5.0, leading to the transfer of Cyt c from mitochondria to cytosol; and the DOX/MPP-DGL escaped from lysosomes and DOX/duplex was delivered to mitochondria. Hence, increasing the amount of Cyt c caused the DNA duplex to become unstable, thereby releasing DOX. It was observed that these

liposomal carriers used for the SR of two chemotherapy drugs, were more effective in killing cancer cells, inhibiting cell growth, and preventing MDR; nonetheless, further research is required to validate this strategy [175].

As mentioned earlier, various organic and inorganic-based nanoplatforms have the potential for effective SR of drug/genes into different cells and tissues. However, in order to design an effective nanoplatform, it is necessary to understand the different properties of various nanomaterials. Table 1 summarizes some advantages and limitations of the various organic and inorganic nanomaterials used in nanoplatforms for drug/gene delivery Sequential release-based technologies

In recent years, various types of technologies have been performed to enable controlled release of therapeutics, as well as the early diagnosis of various diseases, especially different kinds of cancer.

3.8. Titania nanotube (TNT) arrays

Titania nanotube (TNTs) arrays can be fabricated by a low-cost and versatile electrochemical anodization procedure, and have been widely explored as a novel approach to overcome the disadvantages of systemic drug delivery. The TNTs can be synthesized from different substrates such as Ti, Ti alloys, etc, and can have various shapes (plates, needles, etc) [176, 177]. The integration of these arrays with implantable medical devices can create a combination of diagnostic and therapeutic functions into the same device [178]. TNT arrays can serve as DDSs allowing CR due to their biocompatibility, controllable dimensions, the capability of carrying both hydrophilic and hydrophobic drugs at the same time, as well as their chemical stability [176, 179].

Aw *et al.* incorporated TNT arrays into a mycelium polymeric delivery system, which allowed loading of three or more polymeric micelles containing different hydrophilic and hydrophobic drugs, and releasing them in independent pathways without mixing them up. In this study, TNT arrays were synthesized by a self-ordering electrochemistry anodization process in titanium, and were then loaded with two types of micelles; a regular micelle containing two hydrophobic drugs (indomethacin and itraconazole) and an inverted micelle loaded with the hydrophilic drug gentamicin (Fig. 9a). The release profile demonstrated that these three drugs were sequentially released from the two carriers in two separate stages (5 days each) when exposed to the cellular environment (Fig. 9b–d). This system allowed adjusting the ratio of the drug-loaded carriers, to control the amount, release location, and release order of the different drugs (hydrophilic, hydrophobic, anti-inflammatory, antifungal, and antibacterial). This system was proposed to address postoperative care issues (prevent infection and improve healing) relating to bone implants and bone surgery [33].

3.9. 3D printing technology

3D printing technology was originally introduced by Chuck Hull in 1986, and is a rapid and emerging manufacturing technique used to manufacture 3D complex objects with high accuracy using a layer-by-layer assembly procedure based on a computer-aided design (CAD) model [180]. The manufacture of 3D products with precise and controlled architecture, geometry, and shape, with high reproducibility is possible [180, 181]. In recent

years, more attention has been paid to the applications of this technology in medicine and pharmaceuticals. With the advent of 3D printers, the design of high-performance drug delivery arrays and devices with adjustable release kinetics, has been made simpler, for these devices have streamlined the production process of 3D biomedical objects such as multi-active tablets, stents, and tubes [182, 183].

Do *et al.* took advantage of this technology to prepare printed alginate-PLGA tubes (using a coaxial extrusion system) for releasing fluorophores by an SR program. These printed 3D tubes consisted of an alginate shell and PLGA core layers, in which fluorescein was loaded into the alginate shell and rhodamine B into the PLGA core. In this system, fluorescein was released immediately and continued for 24 h, while subsequently, the delayed release of rhodamine B occurred, thus indicating the efficiency of the system in the separate SR of fluorescent dyes. The tubes also showed no signs of toxicity in *in vitro* studies, which proved their biocompatibility for *in vivo* applications [34].

Misra *et al.* also employed this technology to fabricate vascular stents for patients with blocked coronary arteries. The stents were synthesized using poly-l-caprolactone (PCL) and graphene NPs (3D-printed PCL-GR stents), enabling the delivery of niclosamide (nic) and inositol phosphate (IP6) for the prevention of cell growth and anticoagulant activity in pig hearts. The PLC biodegradable polymer is particularly suitable for preparing polymeric implants including stents, due to its low melting point (about 60 °C) and a glass transition temperature of around −60 °C. It also has a relatively lower cost compared to other biodegradable polyesters and is compatible as a filament for the 3D printer. The integration of graphene into the PCL matrices produces a composite that has the appropriate mechanical strength for use in prototype cardiac stents and SR-based DGDSs [35].

3.10. Coaxial electro-spraying

Coaxial electro-spraying is an advanced technique that facilitates the preparation of multilayer encapsulation structures for drug delivery and biological applications. These structures have two feed capillary channels that are important for the development of core-shell carriers for DDSs without requiring surfactants or elevated temperatures [184, 185]. This technique permits the simultaneous encapsulation of hydrophobic and hydrophilic drugs, with no loss of biological activity, for effective delivery at the targeted site [186, 187]. It is also possible to control the material flow rate and synthesis of uniform double-walled microspheres [188].

For instance, Cao *et al.* utilized coaxial electro-spray technology to synthesize two different core-shell carriers (PVP/PLGA and PCL/PLGA NPs) having different features using immiscible and miscible liquids to deliver DOX and combretastatin A4 (CA4). In an acidic medium (pH 6.5), the NPs released the drugs with different release rates: due to the higher affinity of PVP polymer with the hydrophilic core, PVP-DOX/PLGA-CA4 NPs exhibited a faster release rate than CA4-PCL-DOX/PLGA NPs. Other features of this system are described in section 2.3 under the category of core-shell structures [54].

In order to improve wound healing in a controlled manner, Guo *et al.* prepared pH-responsive chitosan–polyethylene oxide/polycaprolactone (chitosan/PEO/PCL) nanofibrous

mats via a coaxial electro-spray technique that could be co-loaded with two drugs (lidocaine hydrochloride, Lid and CUR) and allowed SR. These nanofibrous mats consisted of a chitosan/PEO shell, in which the Lid was loaded into the shell, and the anti-inflammatory CUR was loaded into the PCL core containing acid-sensitive sodium bicarbonate (SB). In the acidic wound environment (pH 5.4), caused by bacterial activity, rapid release of Lid from the shell was triggered due to the protonation of $–NH_2$ on the pH-sensitive chitosan chains, to create rapid pain relief (release rate: 57.43% within 72 h). There was also sustained release of CUR (release rate: 68.24% at 72 h), to reduce inflammation and improve wound healing, as a result of the SB reaction with hydrogen ions and the formation of holes in the fiber mats. This platform allowed adjusting the ratio of the release rates of the two drugs, with a good antibacterial performance [189]. Table 2 summarizes some studies in which different stimuli and different types of carriers have been employed for the SR of drugs/genes.

4. Advantages, Limitations, and Critical Remarks

This section attempts to address some questions about the benefits and limitations of various types of stimuli-responsive DGDSs. We will discuss stimulus-responsive systems, which could be appropriate for the SR of therapeutic agents. We will suggest some possible solutions and strategies to overcome the limitations.

In general, traditional DGDSs, which can carry high concentrations of drug/genes, face challenges such as the instability of the carriers or cargos, and overcoming barriers to reach the target from the bloodstream. Stimuli-responsive delivery systems provide improved control over the spatial-temporal release of the cargo into tissues, compared to conventional systems. As a result, these systems reduce the side effects of common anticancer drugs, reduce damage to healthy organs and tissues, and creates a targeted treatment, especially for cancer treatment [191].

Due to the variations in redox potential in different environments, great attention has been paid to the design of redox-sensitive systems for drug and gene delivery. Tumor tissues have a higher intracellular level of GSH compared to normal tissues [192, 193]. This feature can be used in the design of drug delivery systems with high specificity and efficiency. However, the intrinsic heterogeneity of tumors causes problems with the basic mechanism of redox-sensitive systems.

The application of enzymes as biological stimuli to trigger highly selective drug/gene release is an emerging field in the design of delivery systems. Most enzyme-responsive DGDSs rely on the increased activity of enzymes related to metabolic and biological processes. Also, the use of peptide linkers in carriers designed to mimic the natural enzymatic substrate can effectively release drugs and genes [29]. However, in order to design effective enzyme-responsive systems for clinical applications, factors such as the spatial-temporal pattern of catalytic activity, the effects of possible toxicity, the long-term compatibility with the biological function of the enzyme, and possible release of the therapeutic agent before reaching the desired target, should be considered. Moreover, the use of additional materials,

that are sensitive to other stimuli, such as pH could preserve the carrier intact until it reaches the target.

pH-sensitive delivery systems have been designed in which drug/gene release is precisely triggered by the acidic tumor environment or external environmental conditions. Changes in an acidic environment, trigger the protonation or deprotonation of functional groups, promoting changes in the structure and solubility of the carriers. Decreased side effects and improved drug/gene targeting to specific areas are advantages of these systems [28]. pH-sensitive polymeric carriers can efficiently deliver agents to the tumor microenvironment with decreased side effects. However, different parts of the tumor tissue may show different levels of acidity, and sometimes there is no clear difference between the pH of tumor tissue and the surrounding normal tissue. Designing carriers with improved structural stability against slight pH changes may be an efficient strategy. Polymers containing tertiary amine groups can act as a buffer, so their pH does not change very easily, and they can be used for the design of pH-sensitive carriers [194].

Light-responsive and temperature-sensitive carriers are among the most important smart delivery systems, because of their distinctive properties and also the ability to deliver light and heat froman external source. Despite the fact that these light stimulus-responsive SR systems possess a number of advantages, such as enabling controllable drug release, reducing the side effects of drugs, they also have a number of disadvantages [4, 11]. Light irradiation has limitations related to the power density and wavelength, because any power density above 1 W/cm^2 is harmful to tissue, and not all wavelengths of light are suitable for therapeutic applications. UV light is a case in point, for it is not able to penetrate into deep tissues, and moreover can damage nucleic acids. NIR light can overcome these issues to some extent because it can penetrate into tissues up to 1 cm, and is much less harmful [136, 195]. On the other hand, NIR light does not have sufficient photon energy to allow most photochemical reactions to occur. However, this challenge could be solved by taking advantage of mechanisms, such as rare earth upconversion nanoparticles or two photon absorption chromophores [136, 196].

Temperature-sensitive delivery systems can show tunability of their phase transition temperatures, and a rapid response to thermal changes [197]. Thermosensitive polymers or liposomes can undergo a transition from the gel phase to the crystalline liquid phase after heating, allowing specific drug release in the targeted region [198]. In general, the design of thermosensitive carriers is based on maintaining stability at temperature of 37°C, and then releasing the cargo based on an increase in the temperature to 40–45°C [199]. However, there may be challenges to maintaining the carrier structure, and governing its sensitivity to slight temperature changes. Carrier designed with a 3D structure without any reactive groups, and carriers containing transition metals (stable transition metals including d^0, d^5 and d^{10}), could be used for the synthesis of more stable and safer carriers.

Magnetic targeting uses an external magnetic field in order to control the location of a magnetically responsive drug carrier *in vivo* [200]. The importance of using a magnetic field-sensitive system is due to the lack of magnetic materials within the body, compared to

other stimuli such as light and temperature [26]. Magnetic fields have their own disadvantages such as a relatively high cost compared to other physical methods [195].

In general, internal stimuli-responsive systems (*e.g.,* enzymes and redox) suffer limitations such as low flexibility, however, external stimuli-responsive systems also have their own limitation. For example, the required power density and wavelength of light, or the higher cost of magnetic-responsive systems. Although the use of dual/ multiple stimuli-responsive systems can overcome some of these limitations, the development of more efficient *in vivo* stimuli sensitive platforms requires further effort.

5. Conclusions and future directions

This review article has covered recent research on the development of new types of stimuli-responsive systems and technologies enabling SR-based drug/gene delivery to the tissues and organs of the body, both *in vivo* and *in vitro.* Researchers have long been looking for effective ways to reduce the side effects of highly efficacious drugs, yet do not suffer from their own particular set of additional adverse effects. Many powerful drugs affect both diseased cells and normal cells at the same time. Hence, in the realm of drug delivery, there is a growing demand for innovative drug transfer systems using smart carriers for programmed delivery of one or more drugs or genes. SR-based DGDSs are thus expected to make significant contributions to the future treatment of disease, and cancer therapy in particular.

Cancer cells mostly develop resistance to chemotherapy drugs placing a serious obstacle in the way of cancer treatment. During chemotherapy, the drug enters the cells, but some cells develop specific efflux pumps on the membrane which quickly remove the drugs. Two genes involving in this process are MDR1 and MRP1. If the efflux pumps can be disabled by inhibitors or by gene silencing approaches, the tumor cells can become re-sensitized to chemotherapy drugs, thus improving the efficacy. For instance, using the siRNA technique, the deactivation of gene expression for an efflux pump leads to the re-sensitization of cancer cells to drugs, and the enhancement of chemotherapy. In spite of the promising function of siRNA, it’s uptake kinetics and intracellular localization is problematic; hence, SR-based DGDSs, especially dual/multi-stimuli-responsive DGDSs, can provide sequential transfer of siRNA and the relevant drugs that would otherwise have been pumped out, into the targeted cells, thereby increasing the effectivity of MDR cancer therapy.

Aside from gene therapy and drug delivery in cancer treatment, SR-based DGDSs have also been used for other biomedical applications, such as regeneration and wound healing, in which the SR of therapeutic agents into relevant sites is of vital importance.

In recent years, advanced technologies such as 3D printers and electro-spraying techniques have opened the door to the fabrication of specially designed drug/gene delivery carriers and arrays. These technologies have streamlined the fabrication of carriers that are capable of loading hydrophobic and hydrophilic drugs with the controllable release in different temporal and spatial parameters, thus reducing their toxicity.

Notwithstanding the tremendous opportunities provided by the introduction of smart nanocarriers and microcarriers for SR-based DGDSs, there are still some challenges to overcome. One of these is the toxicity of NPs and their accumulation in the body. It is thus necessary to render the NPs highly biodegradable, but yet be sufficiently stable in the blood circulation. This can be achieved by optimizing the synthesis methods and functionalizing or coating the NPs with polymers that are highly degradable and nontoxic. Another matter of concern that needs to be further addressed, is the nonspecific distribution of stimulant factors, particularly internal stimuli (*e.g.,* high GSH) that may exert off-target effects; and in the case of external stimuli (*e.g.,* UV), the risk of mutation and the long-term damage of cells remain of concern. The heterogeneity of tumors and their variable stages are also among the important issues that should be considered in designing stimuli-responsive DGDSs.

In general, transferring drugs/genes using appropriate nanocarriers that are programmed for the sequential release of cargos has laid the foundation for the more efficient disease treatment. They have the ability to attack diseased cells without damaging surrounding normal cells, together with reducing the side effects of drugs. The expression of the genes involved in MDR can be decreased, reducing overall costs, and enhancing the efficiency of the treatment. Nonetheless, in order to complete the successful bench-to-bedside translation of this innovative technology, further extensive investigations are still required to answer the questions regarding the toxicity and safety of carriers, to devise cost-effective and facile synthesis processes, minimize the side effects and maximize the efficiency of the therapeutic agents. However, in light of the significant progress achieved in the development of safe and cost-effective DGDSs thus far, this field is expected to reach continue to expand in the near future, and eventually to eradicate many diseases such as cancer.

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Highlights

- An in-depth discussion on stimulus responsive sequential drug/gene release systems.
- Mechanistic aspects of sequential cargo delivery systems are discussed.
- Different stimulus factors determining sequential release are reviewed.
- A discussion on recent technologies, advances and limitations is provided.

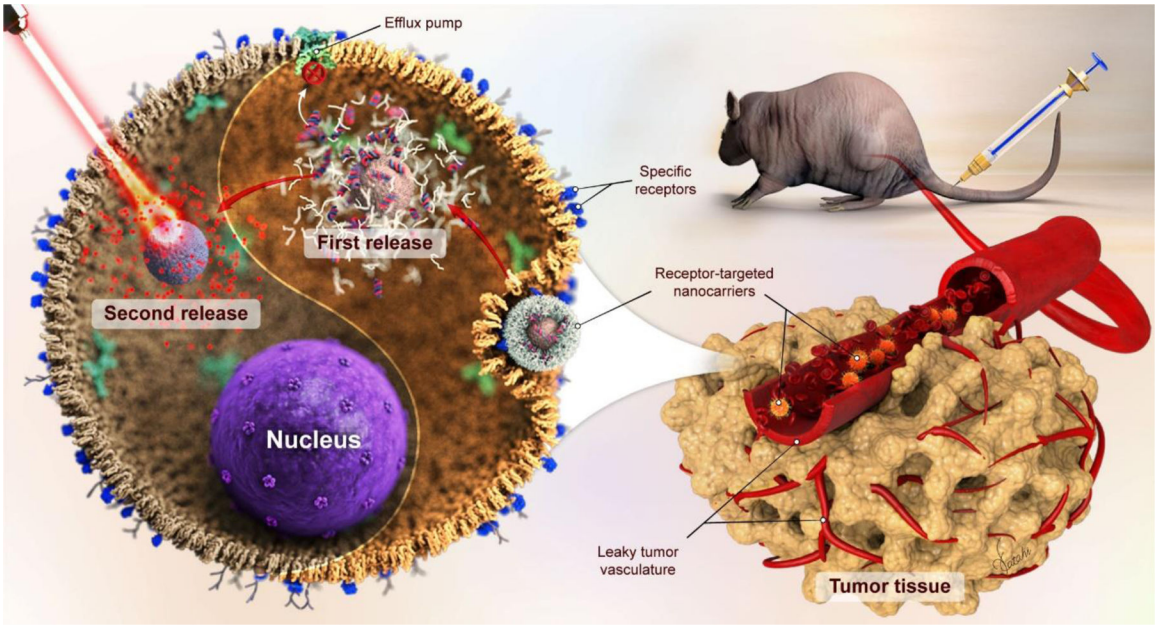

Fig. 1.

Schematic illustration of the effects of stimulating factors in sensitive carriers mediating sequential drug release in a tumor model.

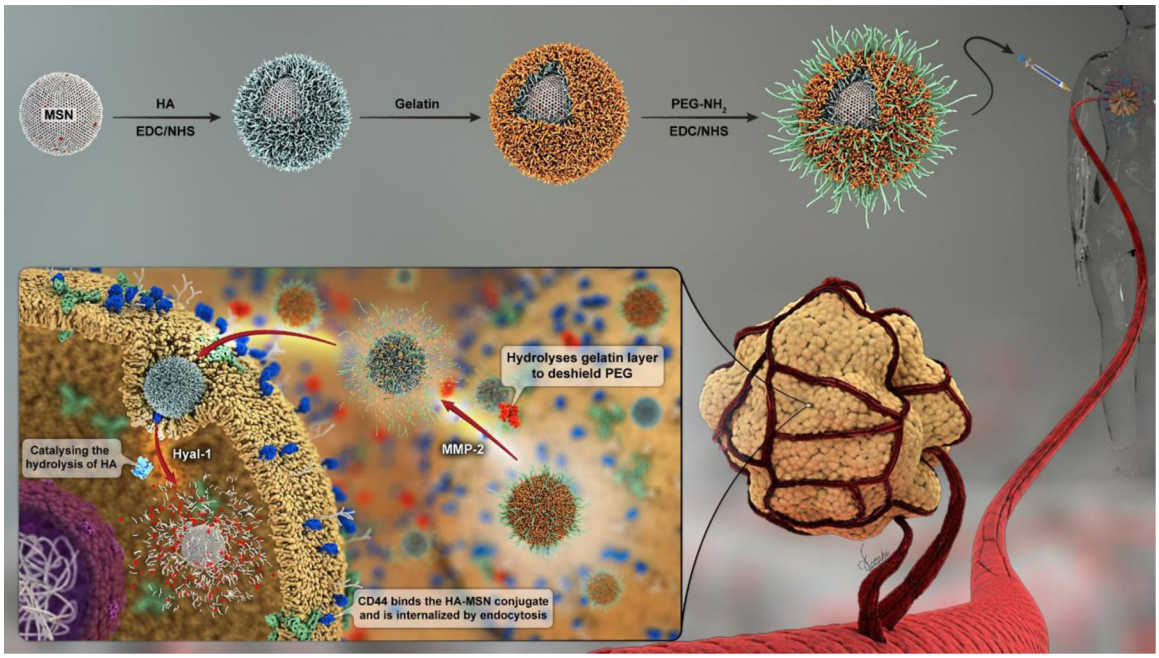

Fig. 2.

Schematic illustration of the CR multifunctional DOX loaded MSN. As depicted MSNs were decorated in a stepwise manner by HA, gelatin, and PEG. MSN@HA was loaded by DOX. In a bienzymatic responsive process the gelatin layer was hydrolyzed by MMP-2, and after HA receptor-mediated endocytosis the MSN@HA/DOX was trapped in the tumor and underwent HA hydrolysis, DOX released in controlled manner [56]. The figure was adapted from Reference [56] and reproduced under the Creative Commons Attribution License, which permits unrestricted use.

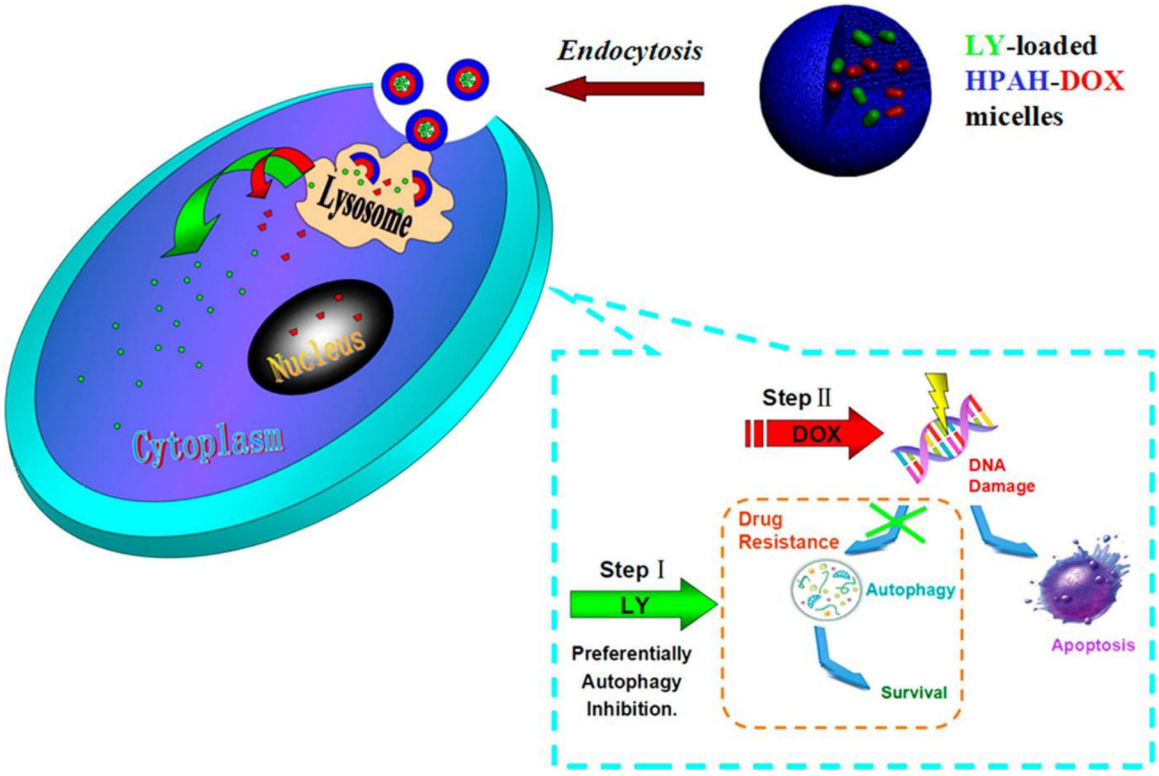

Fig. 3.

Schematic illustration of HPAH-DOX micelles encapsulating LY. In this system, the SR was demonstrated with LY being released faster than DOX, making the tumor cells more sensitive to DOX by inhibiting autophagy. Reprinted (adapted) with permission from [92] Copyright (2020) American Chemical Society.

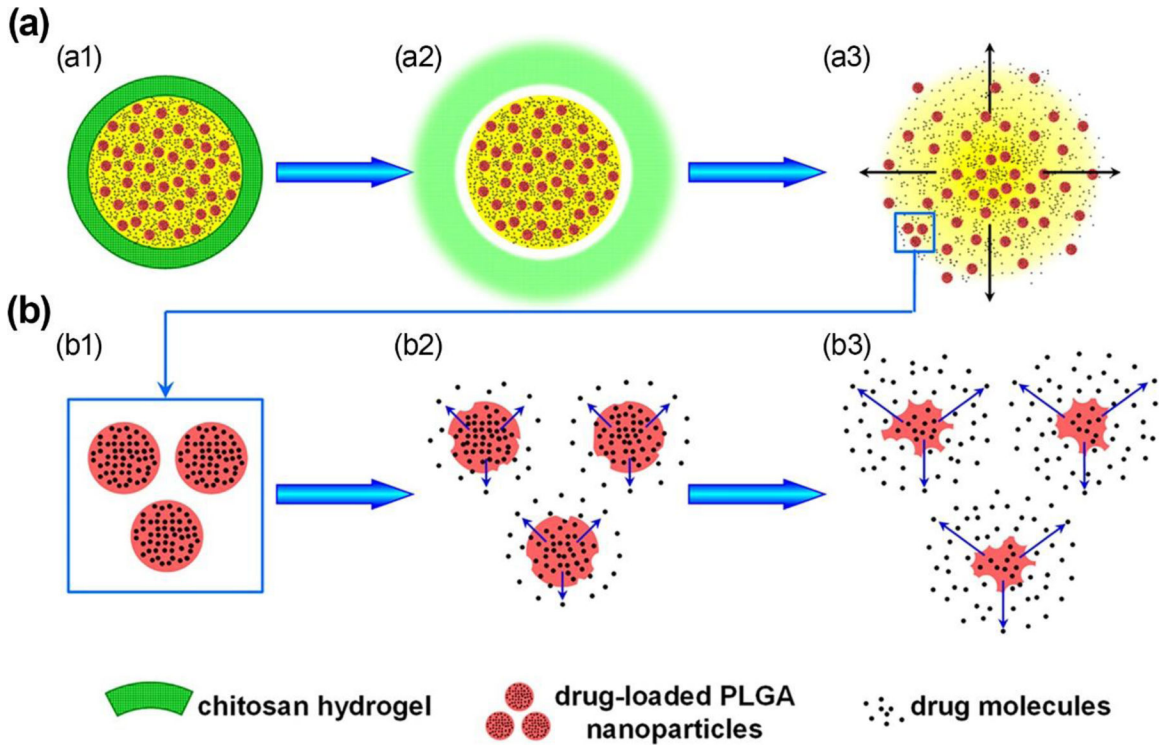

Fig. 4.

Schematic illustration of sequential drug release (CUR & catechin) from pH-sensitive core-shell chitosan microcapsules. a) Decomposition of chitosan shell with burst release of CUR & catechin in acidic conditions; b) Destruction of PLGA NPs and sustained-release of CUR & catechin in two days degradation of PLGA core in acidic conditions Reprinted (adapted) with permission from [20] Copyright (2020) American Chemical Society.

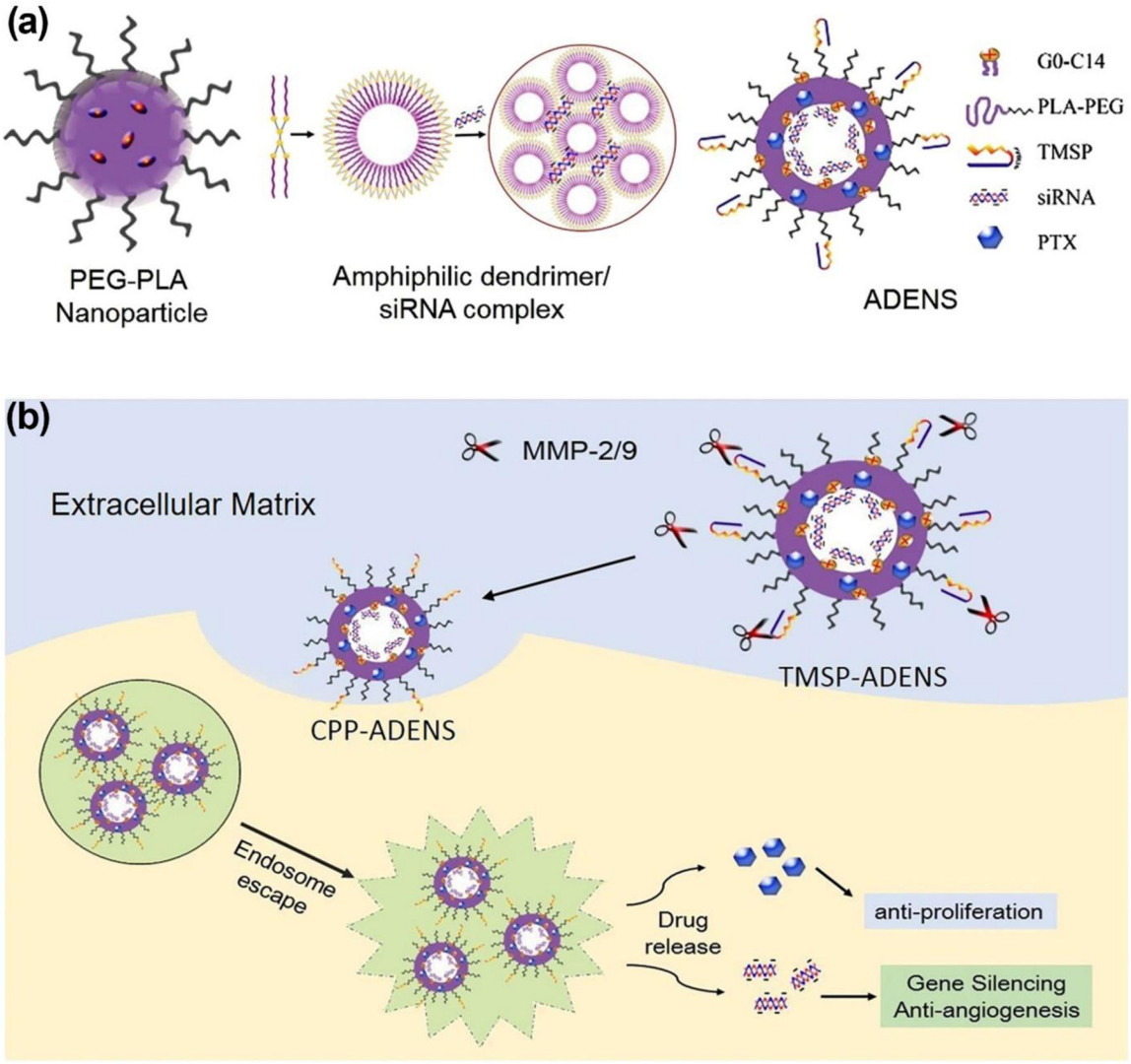

Fig. 5.

a) Schematic illustration of PEG-PLA NPs containing PTX in the PLA core, and the three-layer structure of the ADENS; b) the simultaneous delivery of PTX and siRNA in tumor cells and effect in tumor growth. Open access from Springer Nature[132], no permission needed.

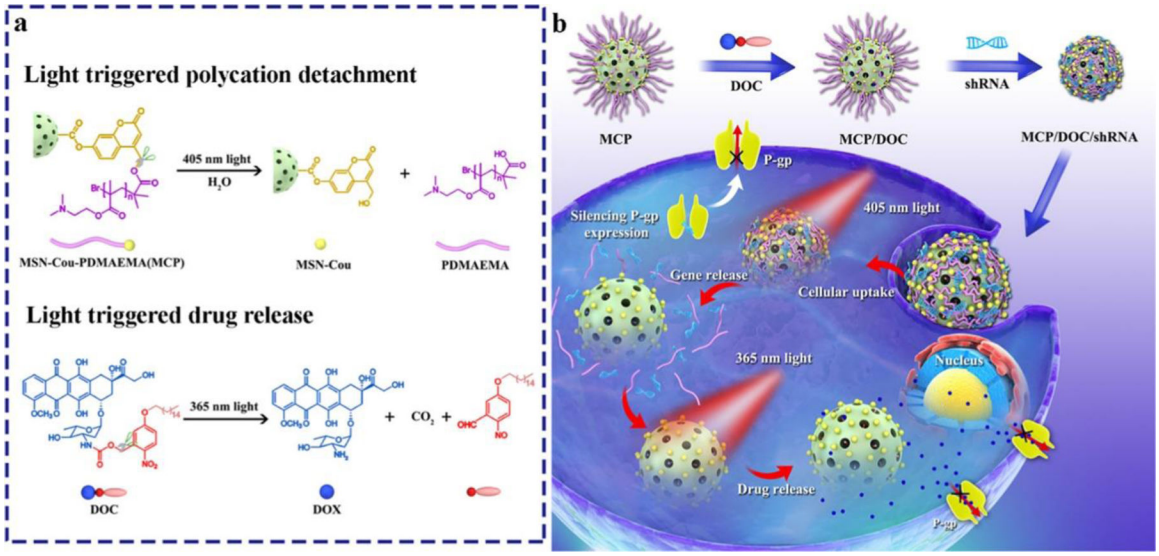

Fig. 6.

a) Schematic illustration of photolysis MCP and DOC under 405 nm and 365 nm, respectively; b) SR of DOX and shRNA using PMSNs regulated by two wavelength light Reprinted (adapted) with permission from [27] Copyright (2020) American Chemical Society.

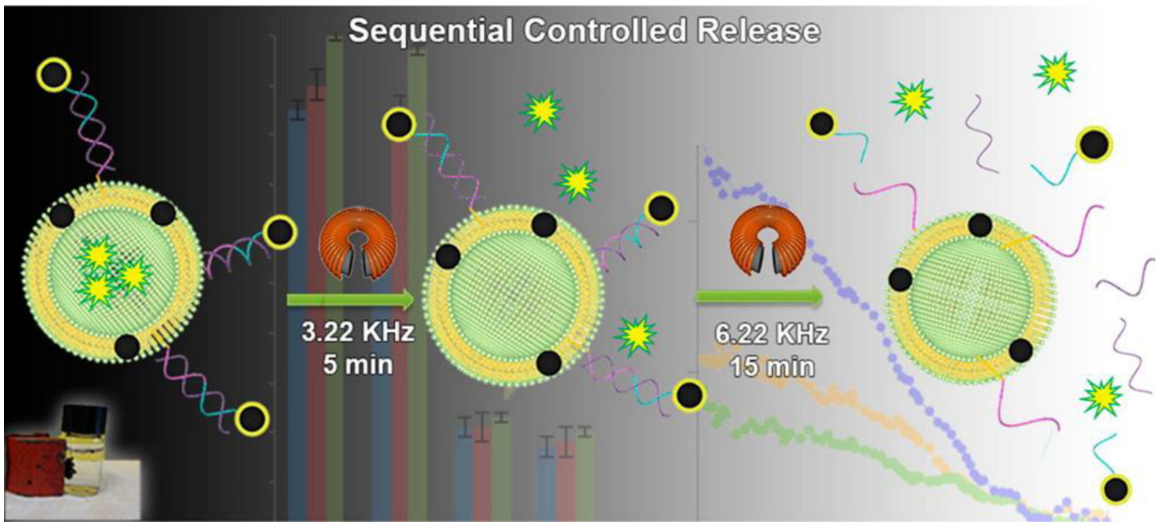

Fig. 7. Schematic illustration of multifunctional magnetoliposomes and the SR of carboxyfluorescein and therapeutic zipper ON.

Carboxyfluorescein was first released after exposure to a 3.22 kHz AMF over a short period of time, subsequently, after the applied of 6.22 kHz AMF, zipper ON (dsDNA hybridized with zipper) was released Reprinted (adapted) with permission from [154] Copyright (2020) American Chemical Society.

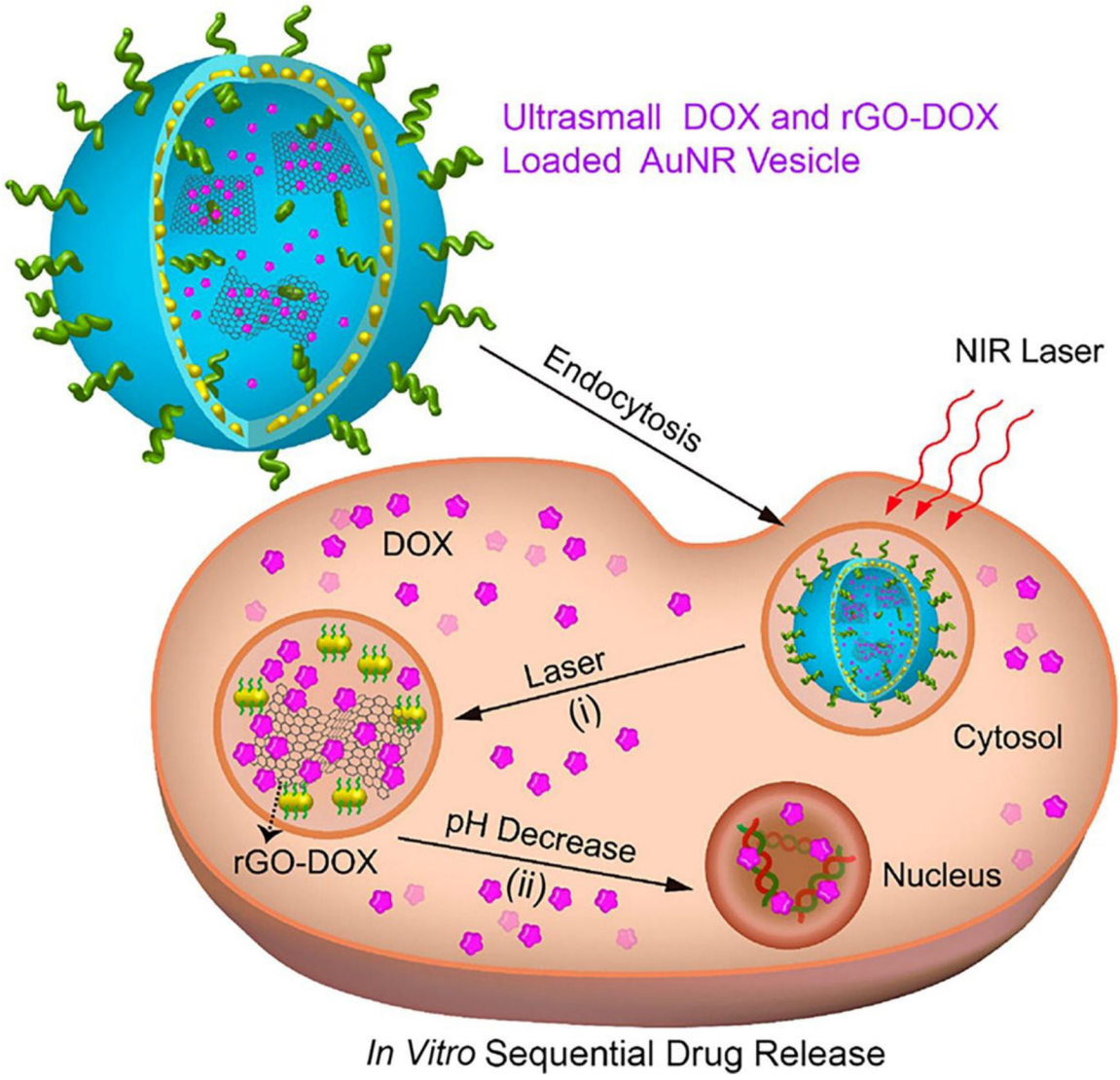

Fig. 8. Schematic illustration of SR DOX releases from rGO-AuNRVe-DOX triggered by both NIR laser irradiation and the acidic environment of cancer cells

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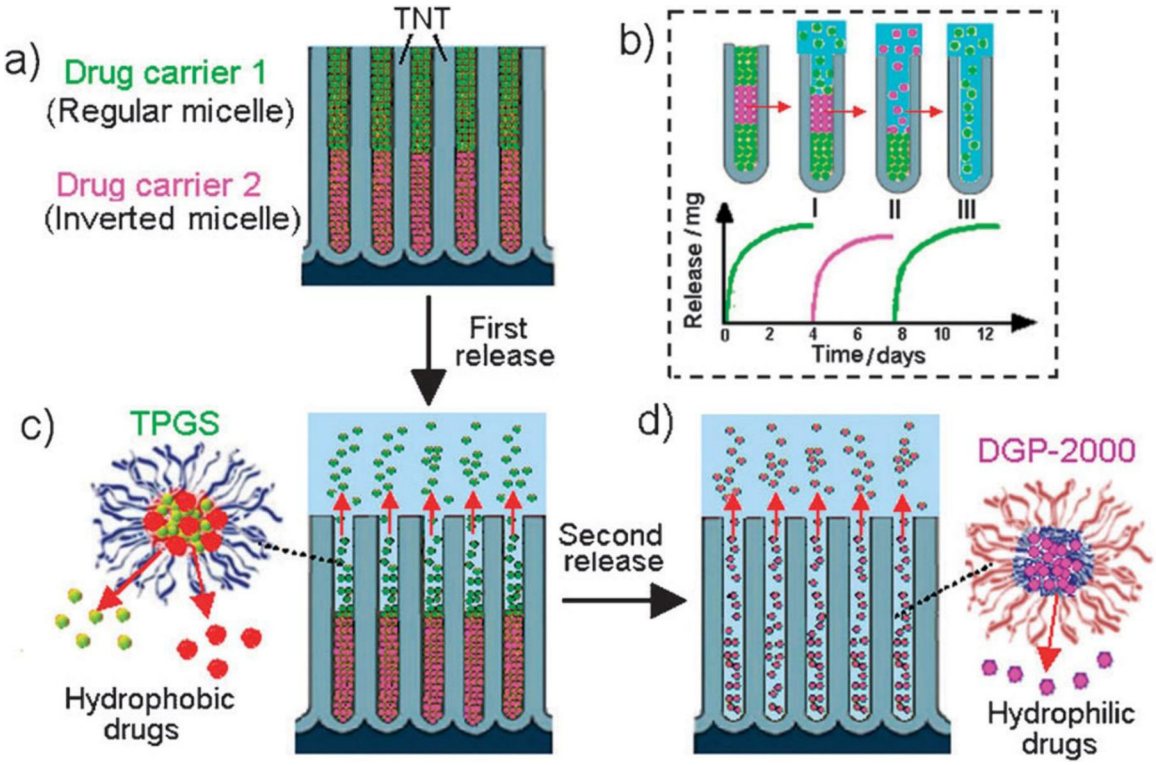

Fig. 9.

Schematic illustration of titania nanotube arrays, polymer micelles, and their role in the SR of multiple hydrophobic and hydrophilic drugs. a) loading of two polymer micelles containing hydrophilic and hydrophobic drugs (indomethacin and itraconazole) in TNT; b) the pattern of sequential drugs release from immiscible layers of carriers; c) and d) showed details of SR [33]. Reprinted with permission from the Royal Society of Chemistry (2020).

Table 1.

Summary of the advantages and limitation of various organic and inorganic nanomaterials applied for drug/gene delivery

Types of Nanomaterials	Advantages	Limitation	Carriers/ drug/gene
Inorganic NPs			
MSNs	• Large surface area • Tunable pore/size morphologies • Ease of functionalization • Controlled release cargo	• Non-biodegradable • *In vivo* toxicity	• PDA-MSN@ZIF-8/DOX, CUR [91] • PMSN/shRNA, DOX [27] • PHMSNs/DOX, Ver [55]
AuNPs	• Biocompatibility • Easy synthesis • Appropriate for photodynamic therapy • Controlled size and surface • High drug loading capacity	• Non-biodegradable • Potential of long-term cytotoxicity and accumulation in the body	• rGO-AuNRVe/DOX [168] • SiO_2@AuNP/HCPT,DOX Bcl.siRNA [30]
MNPs	• Biocompatible • Application in magnetic resonance imaging (MRI) • Hyperthermia treatment • Ease of synthesis	• Non-biodegradable • possibility of toxicity *in vivo* • Long-term tissue damage	• Magnetoliposomes/zipper ON, carboxyfluorescein [154]
Organic NPs			
Polymeric micelles (PMs)	• Biodegradable • High solubility • High stability *in vitro* and *in vivo* • Delivery of poorly soluble drugs	• Short circulation lifetime • Need a surface modification	• LY-loaded HPAH-DOX micelles/ DOX, LY [92]
Chitosan	• Biodegradability • Biocompatibility • Less immunogenic than other polymers • Drug targeting is site-specific	• Insufficient water solubility in alkaline and natural pH	• Glycol chitosan (GC)-based NPs (CNPs)/ DOX, Bcl2.siRNA [99] • CS/PAA@TPGS/PLGA NPS/ VP-16 [100] • Core-shell chitosan microcapsules/ CUR, Catechin [20]
Dendrimer	• Very precise size and shape controllability • Absence of immunogenicity • Water solubility • High degree of branching and agents loading	• Complex synthetic methods • Need for further toxicity and compatibility studies in clinical studies	• Janus PEG-based dendrimers/ BA, PPA [46] • ADENS/ siRNA, PTX [132]
Liposome	• Biocompatibility • Highly efficiency • Overcoming obstacles to cellular uptake • Capacity for loading large hydrophilic and hydrophobic agents • Biodegradable	• May trigger immuno responsive • Cytotoxicity for cationic lipids	• Liposome-cross-linked hybrid hydrogels/ DOX, Cyt [128] • DGLipo NPs Cyclopeptide RA-V, DOX [175]
PLGA polymer	• Biodegradability • Biocompatibility • Long circulation time	• Acidic bio products • Poor drug loading	• CS/PAA@TPGS/PLGA NPs/ VP-16 [100] • Gel-MP / DTX, CA4 [150]

Table 2.

Summary of recent studies investigating SR-based delivery systems based on various types of stimulus factors and types of carriers

Stimuli	Carrier	Drug/Gene	Disease target/ Cells	Advantages	Ref.
pH	PDA-MSN@ZIF-8	DOX, CUR	MCF-7/ADR, MCF-7 cancer cells	The high surface area of core-shell, reduced drug resistance, enhanced synergistic effect of the drugs	[91]
	LY-loaded HPAH-DOX micelles	DOX, LY	oral squamous cell carcinoma (OSCCs)	Integrating chemotherapy with autophagy inhibition; highly effective chemotherapy results	[92]
	glycol chitosan (GC)-based NPs (CNPs)	DOX, Bcl2.siRNA	PC-3 cells	Maximized therapeutic efficacy of DOX due to the incorporation of siRNA into the system	[99]
	CS/PAA@TPGS/PLGA NPs	VP-16	A549/DDP cells	Enhanced cytotoxic effects of the anticancer drugs	[100]
	core–shell chitosan microcapsules	CUR, Catechin	acute gastritis	Simultaneous loading of oleophilic & hydrophilic drugs	[20]
	Janus PEG-based dendrimers	BA, PPA	human umbilical vein endothelial cells (HUVEC)	Nontoxic, biocompatible	[46]
	Nanoparticle (NP) platform	siRNA	HeLa cell	Long circulating in blood flow, efficient gene silencing in solid tumors	[112]
Redox	liposome-cross-linked hybrid hydrogels	DOX, Cyt c	-	Differential release profiles; easily synthesized hybrid system	[128]
	Lipid polymer hybrid NPs	*p53*-mRNA	Hep3B cells, H1299 cells	Increased therapeutic efficacy *in vitro* and *in vivo*	[129]
Enzyme	ADENS	siRNA, PTX	HT-1080, A375 cells	Enhanced cancer combination therapy effect	[132]
	Gelipo	DOX, TRAIL	MDA-MB-231 xenograft tumor animal model	Site-specific SR; highly efficient delivery	[131]
	hydrogel nanocapsules	VEGF, PDGF	diabetic wound healing	Multi-protein delivery; increased vessels; suitable for regenerative medicine delivery & tissue engineering applications	[135]
Light	PMSN	shRNA, DOX	human liver cancer cells	Improved cancer combination therapy effect	[27]
	big & small combo NPs	MB, GM·HCl	HepG-2 cells	Safe and effective chemo-PDT	[144]
	NP_{small}, $NP_{big\&thick}$, $NP_{big\&thin}$	MB, GM, DTX	human PDAC cell lines	Effective chemo-PDT	[145]
Temperature	Gel-MP	DTX, CA4	osteosarcoma	Stepwise release of the drugs; reduced side effects of the antitumor drugs	[150]
Magnetic field	magnetoliposomes	zipper ON, carboxyfluorescein	-	High percentage of drug release at low frequencies	[154]
Dual/multi stimuli	rGO-AuNRVe	DOX	U87MG cancer cells	Excellent accumulation of the hybrid vesicle in the tumor (~65 nm); high loading capacity of doxorubicin (DOX)	[168]

Stimuli	Carrier	Drug/Gene	Disease target/ Cells	Advantages	Ref.
	cage-sphere-like AuNC/ $Fe(OH)_3$-PAA JNP	DOX, DTX	liver cancer	The capability of CT/MR, highest tumor inhibition	[19]
	dual responsive polymeric	P-gp siRNA, DOC	MCF/ADR cells	Increased DOX chemotherapy by P-gp siRNA silence in MDR cancer cells	[29]
	PHMSNs	DOX, Ver	MCF7/ADR breast cancer cell line	Time-dependent SR of the drugs, improved treatment of cancer	[55]
	PAA-ss-MCN	DOX	HeLa cells	Good compatibility; high toxicity of the drug-loaded carriers	[176]
	MSP@P(MAA-Cy)	DOX, TXL	HeLa cells	Low cytotoxicity; programmed drug release	[174]
	SiO_2@AuNP	HCPT, DOX, Bcl.siRNA	Colo-205 cell	Capability of releasing three drugs at different times; enhanced chemotherapy effect	[30]
	DGLipo NPs	Cyclopeptide RA-V, DOX	MCF-7, HeLa, HeLa/MDR cells	Highly effective combination therapy of MDR tumors	[175]
	DCZ-NPs	Zol, DOX	MCF-7 breast cancer cells	Highly effective chemo-thermotherapeutic treatment of breast cancer	[170]
Technologies	titania nanotube (TNT) arrays (polymer micelles)	Indomethacin, Itraconazole, Gentomicine	-	Controllable amount, release location, and release order of the drugs	[33]
	3D printer (Alginate-PLGA tubes)	Fluorophores	human embryonic kidney cell line (HEK293), bone marrow stromal cells (BMSCs)	Efficacious drug treatments	[34]
	3D-printed PCL-GR stents	Nic, IP6	percutaneous coronary intervention	Drug-loaded & nontoxic PCL-GR stents	[35]
	electrosprayed (PVP/ PLGA and PCL/PLGA NPs)	DOX, CA4	B16-F10 melanoma cells, human umbilical vein endothelial cells (HUVECs)	Encapsulation efficiencies>90%; highly effective tumor combination chemotherapy	[54]
	hierarchical 3D multidrug scaffolds	antimicrobial agents (levofloxacin, rifampin, vancomycin)	bone infection	SR of multiple drugs; local bone infection therapy	[190]