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Use of stimulatory responsive soft nanoparticles for intracellular drug delivery

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ABSTRACT

Drug delivery has made tremendous advances in the last decade. Targeted therapies are increasingly common, with intracellular delivery highly impactful and sought after. Intracellular drug delivery systems have limitations due to imprecise and non-targeted release profiles. One way this can be addressed is through using stimuli-responsive soft nanoparticles, which contain materials with an organic backbone such as lipids and polymers. The choice of biomaterial is essential for soft nanoparticles to be responsive to internal or external stimuli. The nanoparticle must retain its integrity and payload in non-targeted physiological conditions while responding to particular intracellular environments where payload release is desired. Multiple internal and external factors could stimulate the intracellular release of drugs from nanoparticles. Internal stimuli include pH, oxidation, and enzymes, while external stimuli include ultrasound, light, electricity, and magnetic fields. Stimulatory responsive soft nanoparticulate systems specifically utilized to modulate intracellular delivery of drugs are explored in this review.

KEYWORDS

nanoparticles, biomaterials, stimuli-responsive, targeted delivery, intracellular drug delivery

1 Introduction

Modern-day drug delivery systems depend highly on targeted delivery strategies where active pharmaceutical ingredients are precisely transported to the intended site of action to achieve the desired therapeutic effect. Nanoparticulate-based delivery systems dominate in targeting drugs to extracellular and intracellular spaces, which helps minimize off-target drug side effects. Nanoparticles possess at least one dimension at the nanoscale (10^{-9} m) and are made up of synthetic and naturally occurring biomaterials [1]. An ideal nanoparticulate system should be biocompatible, biodegradable, non-cytotoxic, and non-immunogenic after *in vivo* administration. In general, nanoparticles can be classified as either "soft" or "hard." Soft nanoparticles are made up of materials containing an organic backbone, such as lipids and polymers, while hard nanoparticles are made up of inorganic materials [2]. Soft nanoparticles are flexible in nature, which refers to particle surface rigidity; soft materials have the capacity to change shapes as they transverse throughout the body [3, 4]. Soft nanoparticles can deeply penetrate porous tissues and allow for better drug delivery to tumors [4, 5]. These characteristics contrast with hard nanoparticles. Hard nanoparticles are still made from an organic backbone. However, the significant difference is that they cannot readily change size and shape *in vivo*, hindering capillary extravasation and lymphatic system penetration [6]. Hard nanoparticles are often rigid and possess a crystalline structure; however, in some instances, they may also be inorganic particles that may be coated by an organic material [6]. Most hard nanoparticles, in general, are composed of inorganic materials and have a solid core structure. Of note, lipid and polymeric nanoparticles have proven superior to inorganic nanoparticles in terms of biocompatibility and biodegradability.

Figure 1 depicts some of the most widely studied soft nanoparticle delivery systems, including micelles, liposomes, polymersomes, polymeric nanospheres, and dendrimers [7]. These nanoparticles have been utilized as vehicles for the targeted and controlled delivery of various agents, including small and large-molecule therapeutics, genes, and diagnostic imaging agents [8]. Polymeric nanoparticles are made up of either natural or synthetic polymers. The most commonly used polymers in nanoparticle formulations include polylactide (PLA), polycaprolactone (PCL), poly(lactide-co-glycolic acid) (PLGA), polyethylene glycol (PEG), chitosan, and hyaluronic acid. Lipid-based nanoparticles are usually made up of a mixture of lipids containing phosphatidylcholine, cholesterol, PEGylated lipids (such as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol)), acetyl palmitate, stearic acid, ionizable lipids (such as SM-102 and ALC-0315), and cationic lipids (such as 1,2-dioleoyl-3-trimethylammonium propane (DOTAP)) [9, 10]. On the other hand, amphiphilic copolymers or lipids, surfactants, and proteins are commonly utilized to prepare micelle formulations [11–13]. Of all these delivery systems, lipid nanoparticles are widely used in clinics and approved by the Food and Drug Administration (FDA) for several biomedical applications [12].

Multiple disease states can benefit from intracellular targeting therapeutics; cancer currently has the most utility for intracellular release profile therapeutics. In addition to traditional systemic

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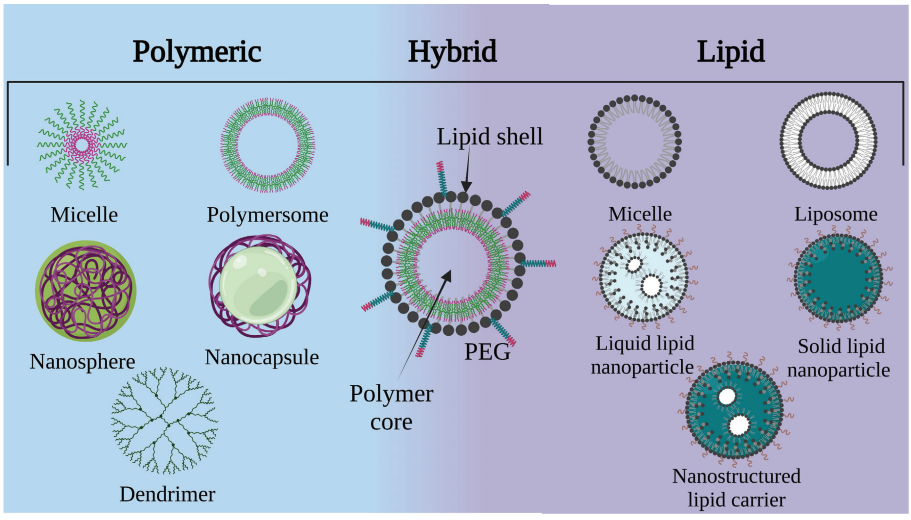


Figure 1 Different classes of polymeric and lipid nanoparticles. Hybrid nanoparticles also contain polymeric cores and an outer lipid shell. Figure created in BioRender.com.

therapeutics, intracellular release-focused nanoparticle formulations are explicitly made for prophylactic and therapeutic vaccines [14]. Regarding the route of delivery, all the FDA-approved intracellular targeting nanoparticle delivery systems utilize the parenteral route of administration. Doxorubicin (DOX), an anthracycline chemotherapeutic agent, was first approved in 1995 with a liposomal delivery system formulated with surface PEG coating and is the most widely used doxorubicin formulation [15]. In addition to doxorubicin, other FDA-approved chemotherapeutic liposomal nanoparticle formulations include vincristine, irinotecan, mifamurtide, daunorubicin, cytarabine, and cisplatin [14, 15]. Paclitaxel, a chemotherapeutic, is not formulated as liposomes but as an albumin-bound nanoparticle [15]. Other nanoparticle delivery systems are being studied, such as polymeric micelles and dendrimers, but liposomal formulations are still the most prevalent [16]. Nanoparticle delivery systems can also be utilized as theranostics in various cancers; it is advantageous to use nanoparticles that can provide both a diagnosis and a treatment for multiple disease states.

Interestingly, these theranostic applications, specifically with cancer, utilize intratumoral delivery while capitalizing on intracellular stimulatory responses, particularly external stimuli such as photodynamic therapy and photothermal therapy, allowing for spatiotemporal precision [17]. Another application of intracellular delivery of theranostic agents includes 5-aminolevulinic acid (ALA). This endogenous peptide may be used to detect solid tumors through photodynamic diagnosis and provide photosensitizer treatment in the photodynamic therapy [18]. ALA enzymatically gets converted to a photosensitizer, protoporphyrin IX in the mitochondria of tumor cells, and ALA within nanoparticle formulations can better escape the endolysosomal pathway, effectively enhancing its mitochondrial targeting [19].

Outside of cancer diagnosis, nanoparticles are also used therapeutically for other indications. For pain management, there are liposomal formulations for bupivacaine delivery and morphine sulfate [12, 14]. The mechanism of action for bupivacaine includes binding to intracellular voltage-gated sodium channels. The liposomal bupivacaine formulation has the advantages of extending the long duration of action of bupivacaine while in the cell and decreasing central nervous sytem (CNS) toxicity risks [20]. For infectious diseases, liposomal amphotericin B is widely used to treat severe fungal infections [14]. This liposomal formulation is used to treat not only extracellular fungal infections but intracellular fungal infections as well, which historically are more challenging to treat [21]. Additional modalities that capitalize on intracellular delivery include vaccines. Most recently, mRNA vaccines have gained tremendous attention for their ability to protect against the SARS-COV-2 virus. mRNA vaccines also have applications outside infectious disease indications and can be used in various treatments where immunomodulation is needed [22]. The various formulations for mRNA vaccines consist mainly of lipid nanocarriers which not only encapsulate mRNA but also protect the mRNA from degradation *in vivo* and transport it precisely to the cytoplasm of the cells [22]. Numerous cargo loads can be delivered in addition to mRNA, such as recombinant DNA, surface antigens, and virally encoded proteins [23]. Some of the nanotechnology-based vaccines that have already been approved include Recombivax®, the hepatitis B vaccine; Gardasil®, the HPV vaccine; and, most recently, Spikevax® and Comirnaty® COVID-19 vaccines [23, 24]. More research is being done to develop nanoparticle delivery vaccines for influenza, rotavirus, SARS, and HIV [23, 25]. In addition to lipid-based mRNA vaccines, three novel protein-based nanoparticle vaccines, including the recently approved Novavax, are also being studied [24].

Nanoparticles have been increasingly prevalent in emerging biomedical applications. Multiple nanoparticle formulations are routinely used in clinical care; these therapeutics are mentioned above. This review will explore how soft nanoparticles are currently being studied for intracellular delivery using stimulatory-responsive materials. These delivery systems must first undergo cellular internalization, which may be accomplished through various endocytic mechanisms. Additionally, cell-specific and subcellular targeting are highly sought-after characteristics for additional precision. The stimulatory responsive nature of these biomaterials has been studied in multiple indications utilizing a wide range of carrier systems and payloads. Internal stimuli tend to be the most prominently used; these internal stimuli-responsive carriers rely on the heterogenicity of physiological spaces. Additionally, external stimuli may trigger drug release, which depends on exogenously applied forces. In general, using stimulatory-responsive soft nanoparticles allows for intracellular drug delivery, providing a more precise and kinetically favorable release profile of therapeutics.

2 Cellular internalization mechanisms and subcellular targeting of nanoparticles

For most chemotherapeutic-loaded nanoparticles, the nucleus is a

primary target for the intracellular delivery [26]. Some of the various intracellular targets are explained extensively by Andraos and Gulumian [26]. Figure 2 depicts that these intracellular mechanisms often target the cell's nucleus, mitochondria, or lysosomes within a cell.

Nuclear targeting includes direct passive entry of chemotherapeutics through Brownian motion, direct DNA damage, and cytokinesis arrest, which leads to mitotic phase arrest. Additional mechanisms include inducing oxidative damage, which impairs DNA repair mechanisms, and nuclear envelope conformational changes through forming nuclear folds [26]. Not only is the nucleus an intracellular target for many anti-cancer drugs, but the mitochondria are as well. Mitochondrial drug delivery has multiple mechanisms; these include voltage-dependent anion channel permeation and cell death caused by reactive oxygen species bursts. Mitochondrial depolarization is the mechanism that disrupts the electron transport chain leading to reactive oxygen and, ultimately, cell death [26, 27]. It has also been demonstrated that mitochondrial stimuli such as alkaline pH, high reactive oxygen species (ROS), and high temperature could be leveraged for the mitochondrial targeting [27].

For targeting the lysosome, mechanisms include causing alkalization of the lysosome and subsequently inducing lysosomal membrane permeabilization. Other mechanisms include Fe^{3+} burst, lysosomal swelling due to the aggregation of nanoparticles within the lysosome, reduction in transcription factor EB resulting in increased autophagosomes, and finally, reactive oxygen species causing apoptosis [26]. The primary target mechanism for the Golgi and endoplasmic reticulum (ER), which interact together, is apoptosis from the ER stress signaling pathway [26]. Vaccines have a different intracellular mechanism than organelle-based mechanisms; vaccines utilize cytosolic release, which works differently than organelle targeting. First, the nano-formulations must be internalized by the cells, this may happen through either phagocytosis or pinocytosis pathways, and they must then undergo an intracellular trafficking [28, 29]. An additional step before cytosolic release is that some nanoparticles must deal with potential localization in endosomes and then subsequently endosomal/lysosomal escape [29, 30]. This endosomal escape can occur through destabilization of the endosomal/lysosomal membrane, resulting in the leaching of particles or particle-encapsulated payloads into the cytosol [29]. The significant mechanistic aspects to consider for cytosolic and organelle release revolves around the impact of stimulatory-responsive nanoparticles as delivery vehicles [29].

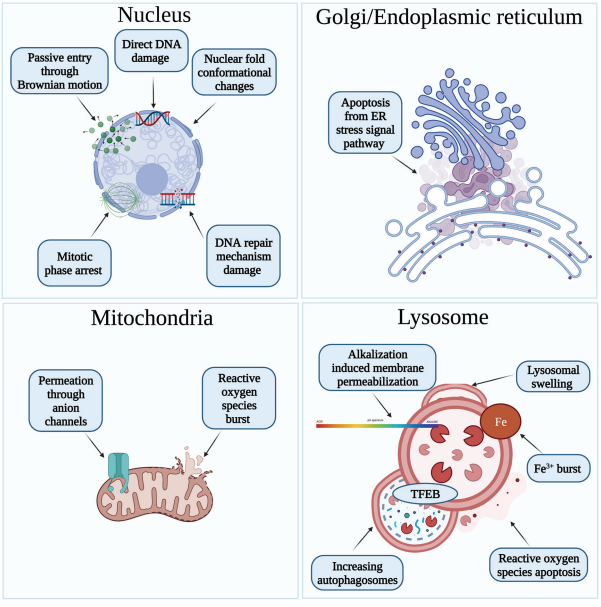


Figure 2 The various mechanisms utilized by nanoparticles for targeting intracellular organelles. These are most commonly the nucleus, endoplasmic reticulum, mitochondria, and lysosome. Figure created in BioRender.com.

While this review focuses mainly on non-targeted approaches, a classification of nanoparticles referred to as third-generation nanoparticles can achieve subcellular and organelle-level targeting [31]. This degree of targeting has yet to be achieved fully and should be researched further [32, 33]. While subcellular targeting is not entirely achievable yet, cell-specific targeting can be achieved through surface modifications by adding targeting ligands [33]. These targeting ligands, however, do not address the challenges and mechanisms of overcoming lysosomal escape once internalized [33]. After internalization, these nanoparticles can undergo their triggered therapeutic release.

Nanoparticles may utilize various cellular internalization mechanisms; these mechanisms vary based on cell type. Figure 3 depicts the six pathways that nanoparticles undergo endocytosis. The most utilized pathway is clathrin-mediated endocytosis; both polymeric and lipid nanoparticles almost exclusively utilize this method of endocytosis. This process occurs with cytosolic endocytic coat proteins covering a portion of the plasma membrane, forming a clathrin-coated pit that encapsulates the nanoparticle to form a clathrin-coated vesicle, which typically can be up to 200 nm in size. Through scission via a dynamin-dependent cleavage and actin polymerization, the vesicle breaks from the plasma membrane where the coat proteins release the vesicle, which now holds the bound plasma membrane proteins and encapsulated nanoparticles [34–36]. Once invaginated, the vesicle sheds the clathrin coat, which binds with early endosomes and follows the endo-lysosomal pathway for the eventual cargo release [35]. Caveolae endocytosis is a clathrin-independent and dynamin-dependent process [37]. This pathway involves membrane proteins such as caveolin and cavin proteins lining the plasma membrane invagination and working with cytosolic accessory proteins. Once the designated nanoparticle reaches the receptors on the plasma membrane, these membrane and accessory proteins activate signaling pathways, allowing the formation of caveosomes, which fuse with early endosomes. Caveosomes are neutral in pH, escape lysosomal fusion, and degradation, and can encapsulate various types of coated nanoparticles; however, they are smaller than clathrin-coated vesicles, limiting the drug internalization [35, 36].

There are other clathrin-independent mechanisms as well. An example is dynamin-dependent fast endophilin-mediated endocytosis (FEME), activated by G protein-coupled receptors, tyrosine kinase reactors, and cytokine receptors. The priming of endophilin at the plasma membrane allows the formation of tubulo-vesicular carriers very rapidly, which helps carry nanoparticles to the early endosomes [38, 39]. Similarly, the clathrin-independent carriers/glycosylphosphatidylinositol-anchored protein-enriched early endocytic compartment (CLIC/GEEC) endocytosis utilizes tubulo-vesicular carriers to transport nanoparticles to endosomes. Unlike the FEME pathway, the CLIC/GEEC mechanism is regulated by the actin regulatory complex ARP2/3 and the small GTPase CDC42 [40]. Macropinocytosis activated via actin polymerization leads to the plasma membrane remodeling to form ruffles extending from the membrane to engulf large quantities of extracellular fluid [41–43]. Phagocytosis is an additional mechanism in which exogenous material may be endocytosed and is triggered by ligand-receptor recognition, which then leads to actin polymerization and membrane movement. This movement causes the membrane to extend outward while engulfing the targeted particles [41].

An important quality that nanoparticle carrier systems should

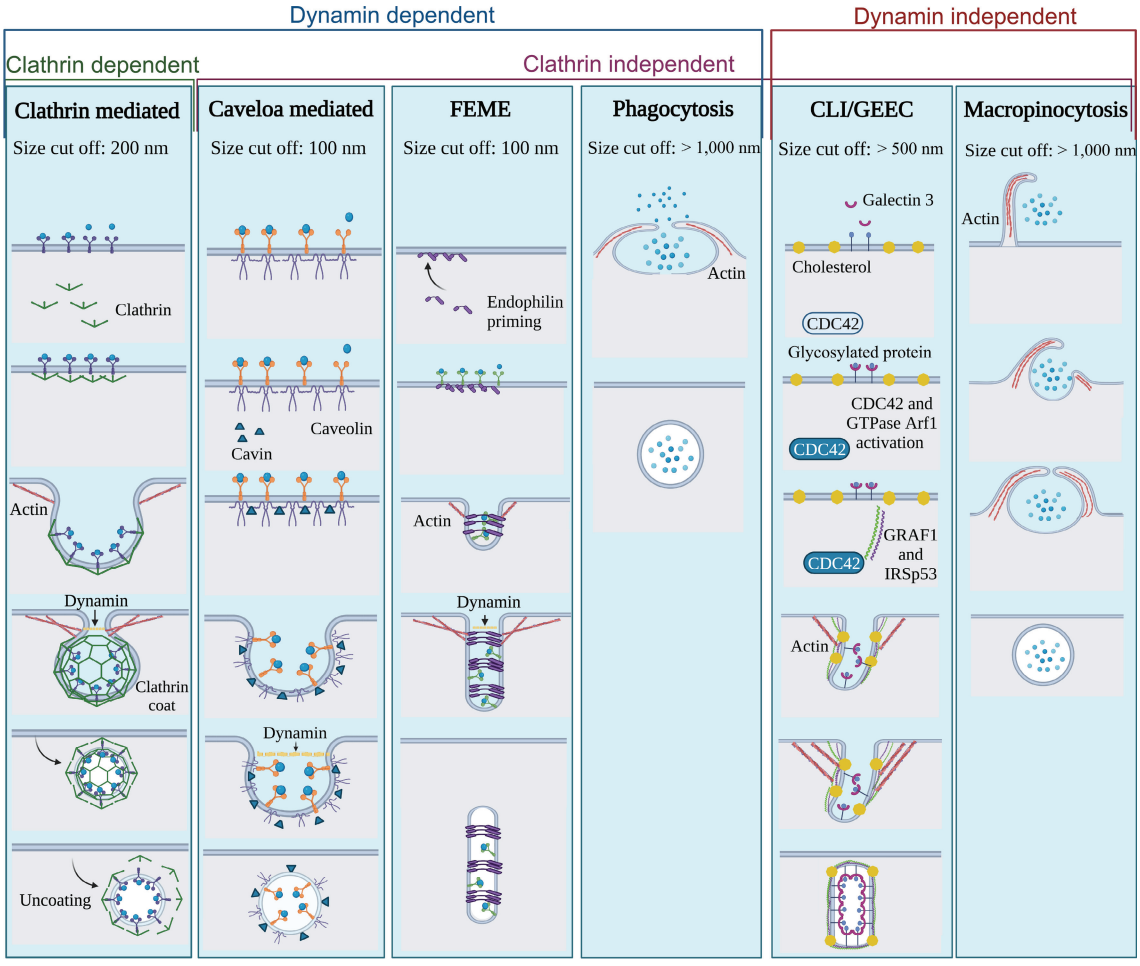


Figure 3 Schematic representing pathways that nanoparticles may undergo cellular internalization. Clathrin-dependent endocytosis is dynamin-dependent, and caveloa, FEME, and phagocytosis are also dynamin-dependent. Dynamin and clathrin-independent endocytosis includes CLI/GEEC and macropinocytosis. Each pathway has limitations for how large a foreign molecule may be to be adequately internalized. Figure created in BioRender.com.

possess is the functionality to release drugs into the subcellular compartments once internalization into the cell is achieved. Nanoparticles rationally engineered for intracellular targeting show benefits such as increased bioavailability and site-specific delivery of drug cargo by avoiding the mononuclear phagocytic system (MPS), improved cellular internalization via cell-specific surface modifications, and stimulatory responsive drug payload release at precise intracellular spaces [44]. There is, however, still the limitation of the imprecise release of drug cargo from nanoparticles while in intracellular spaces. Nanomedicines are continuously undergoing refinement for enhanced drug delivery in response to biological barriers and tailored drug release. A one-way imprecise intracellular release can be addressed is through nanoparticle formulations that respond to physiological (internal) or external stimuli. Intracellular drug delivery systems are especially advantageous for various pharmaceutical indications, with anti-cancer therapies being one of the most prolific [44, 45]. Besides traditional drug molecules, other molecules that rely on intracellular delivery include therapeutic proteins, siRNA, mRNA, and DNA [45]. The therapeutics that benefit from this intracellular targeting most often utilize nuclear targeting or cytoplasmic release but may also target other cellular organelles such as the lysosome, mitochondria, and endosome [46]. The nucleus, one of the most desirable intracellular targets, especially for anti-cancer therapeutics, is targeted due to the disruption of DNA synthesis or through direct DNA damage, which can lead to increased efficiency in the cancer cell eradication [47, 48].

As nanomedicine research has expanded, more disease states, aside from cancers, have been examined for intracellular targeting drug development in inflammatory diseases, infection control, pain management, and immune modulation [14, 30]. For mitochondrial targeting, cationic lipids, peptides, and small molecules are the most widely studied; the mitochondria are worth targeting due to the extensive nature of the organelle. The mitochondria are involved in energy production and cellular respiration while also regulating calcium ion homeostasis, creating reactive oxygen species, and playing a role in cellular apoptosis [49]. Potential mechanisms by which mitochondrial targeting agents act include alterations to the mitochondrial membrane polarity and pore permeability, as well as inhibition of metabolic pathways [49]. On the other hand, lysosomes should not be overlooked entirely; they can also serve as therapeutic targets. Lysosomes are involved in cellular catabolism and various targeting mechanisms such as pH alterations, mTOR inhibition, and autophagy inhibition [50].

3 Stimulatory responsive drug delivery

A significant limitation to intracellular release is the nonspecific release of a drug; this can lead to inadequate drug accumulation and irregular release kinetics. The ability to control the location or timing of payload release from nanoparticles is highly sought after. The choice of biomaterial is essential for the nanoparticle to be responsive to internal or external stimuli (Table 1) [51].

The nanoparticle must retain its integrity and payload in non-targeted physiological conditions while responding to intracellular environments where payload release is desired. Multiple internal and external factors could stimulate the intracellular release of

Table 1 Different stimuli-responsive delivery systems for intracellular release of therapeutics

Stimuli	Carrier system	Payload	Disease state	References
pH	6-Octadecylimino-hexane 1,2,3,4,5-pentanol and isonicotinic acid octylidene-hydrazide	Isonicotinic acid octylidene-hydrazide	Latent tuberculosis	[52]
	Hydroxyethyl starch and Pro-His-Ser-Arg-Asn peptides	Smoothened agonist activator of sonic hedgehog signaling	Ischemic stroke	[53]
	Poly(ethylene lactide)	Camptothecin metformin	Type 2 diabetes mellitus and malignant triple negative breast cancer	[54]
	Poly(D,L-lactic-co-glycolic acid)	Paclitaxel NaHCO3	Restenosis after carotid artery injury	[55]
Redox	Hyaluronic acid conjugated curcumin and d-α-tocopherol acid polyethylene glycolsuccinate	Dasatinib	Hepatocellular carcinoma	[56]
	Chitosan oligosaccharide-ss-hydrophobic curcumin conjugate	Docetaxel	Malignant gliomas	[57]
	Hyaluronic acid derivative was constructed to conjugate with cationic siR-93C@PAMAM	KRAS siRNA	Non-small cell lung carcinoma	[58]
	Polyethyleneimine-tocopherol hydrogen succinate-dithioglycollicacid and hyaluronic acid-QU	Paclitaxel/quercetin	ER + breast cancer (MCF-7)	[59]
	Thiolated vitaminE-PEG1000-succinate	Docetaxel/cetuximab	Lung cancer	[60]
Enzymatic	Albumin-binding maleimide	Paclitaxel	Stage 4 breast cancer (4T1)	[61]
	Hyaluronic acid-cystamine-docosahexaenoic acid - chlorin e6	Docetaxel	ER + breast cancer (MCF-7)	[62]
	1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-poly(ethylene glycol) with NAD(P)H: quinone oxidoreductase-1 (NQO1)	Paclitaxel/merocyanine	Lung cancer	[63]
	Hyaluronic acid-acetal-PTX prodrugs chaperoned by αPD-L1 and metalloproteinase-9-responsive outer shells	Checkpoint inhibitor anti-PD-L1 antibody and paclitaxel	Stage 4 breast cancer (4T1)	[64]
	PEG-poly(b-aminoester) micelles coated with hyaluronic acid	Thioridazine	ER + breast cancer (MCF-7)	[65]
Ultrasound	Maghemite-containing PNPs poly(ethylene glycol)/poly(D,L-lactide-co-glycolide)-RegaCP peptide	Rega20mer peptide sequence and maghemite	Pancreatic cancer	[66]
	Poly(D,L-lactide-co-glycolide-COOH)	Chlorin e6 perfluoropentane and docetaxel	Ultrasound imaging and treatment for metastasis breast cancer	[67]
	Human serum albumin	Indocyanine green	Cancer phototheranostics	[68]
	Perfluoropentane/C9F17-PAsp/miR-122/poly(glutamic acid)-g-MeO-poly(ethylene glycol) ternary nanodroplets	microRNA-122	Hepatocellular carcinoma	[69]
Electrical	Poly(D,L-lactide)	Curcumin	Colon cancer	[70]
	Dextran, hexamethyleneDiisocyanate and aniline tetramer	Dexamethasone and indomethacin	Inflammation	[71]
Thermal/ magnetic	Polypyrrole shell and the hyaluronic acidmodified in the shell	Docetaxel	Stage 4 breast cancer (4T1)	[72]
	Poly[4,4-bis(2-ethylhexyl)-cyclopenta[2,1-b;3,4-b′]-dithiophene-2,6-diyl-alt-2,1,3-benzoselenadiazole-4,7-diyl]	Oxaliplatin	Colorectal cancer	[73]

drugs from nanoparticles. For internal stimuli-responsive formulations, it is imperative to understand the various intracellular environments and how they may change in pathological situations. The intracellular environments that target drug response include pH, redox, and various enzymatic involvements. For example, if a nanoparticle's desired target is to release the payload within an acidic environment, there should theoretically be minimal to no release at normal physiological pH. Limiting the drug release at physiological pH allows an adequate drug concentration to reach the site of action.

In contrast to internal stimuli for drug release, external stimuli may also be used. These external factors may include ultrasound (US), electric, light, and thermal/magnetic stimuli [74]. The same concept of tuning the nanoparticle for payload release with certain internal stimuli also holds true for these external factors. In addition to one stimulus, multiple stimuli can be utilized to control the release of therapeutic agents from the nanoparticles.

4 Internal stimuli

4.1 pH

One of the most highly researched stimuli-responsive drug release mechanisms focuses on pH variation. This is because of the inherent pH differences in extracellular and intracellular environments (Table 2).

Within cellular organelles, specifically the lysosome, pH differs from the cytosol leaving additional opportunities for controlled drug release from the moment of entrapment into the endolysosomal pathway, which has an acidic pH and eventually escapes into the cytosol [75].

One mechanism for pH-responsive drug release relies on ionizing various functional groups, such as amines and carboxyl acids, leading to a proton sponge effect. This increased ionization on the nanoparticle surfaces causes an influx of water to enter the endolysosomes, releasing nanoparticles from the vesicles as the wall of the organelle loses its integrity [75]. Le et al. synthesized a dextran-coated nanoparticle consisting of polymer poly(β-amino ester)-guanidine-phenylboronic acid (PBAE-G-B). PBAE-G-B responds to low pH environments through the pronation of its tertiary amines, causing the polymer to become more hydrophilic to promote drug release. The nanoparticles were encapsulated with rifampicin to treat antimicrobial-resistant infections. Interestingly, these nanoparticles showed significant efficacy in both gram-negative and gram-positive pathogen models and eliminated biofilms and intracellular infections [76].

Recently, Gou et al. utilized the method of escape from the endolysosomal pathway with hybrid polymeric nanoparticles synthesized with polyethyleneimine-tocopherol hydrogen succinate-dithioglycollic acid (PEI-TOS-SS) with hyaluronic acid-

Table 2 pH in various physiological spaces

Physiological space	pH
Extracellular/circulatory system	7.4
Cytosol	7.0–7.4
Early endosome	6.5
Late endosome	5.5
Lysosome	4.5
Tumorigenic microenvironment	5.5–7.0

quercetin (HA-QU), which is pH sensitive via β-carboxylic amide bonds. Excitingly, significant cellular uptake of the polymeric particles loaded with fluorescent marker C6 at 4 h was observed in MDA-MB-231/DOX cells with an escape from lysosomal compartments to the cytoplasm significantly at 6 h, which they attribute to the proton sponge effect [59]. Furthermore, when loaded with an anti-cancer drug, paclitaxel (PTX), these nanoparticles showed a 180-fold increase in intracellular PTX concentrations compared to a free drug.

Another method of pH responsiveness involves the incorporation of acid-labile bonds onto the nanoparticles, which upon exposure to the acidic environment, induces physiochemical changes, thereby releasing the payload. One such example includes the shedding of the responsive nano-carrier outercoat made up of poly(ethylene glycol); this, in turn, allows for the active therapeutic payload to subsequently escape (Fig. 4) [75, 77, 78]. For instance, Liu et al. synthesized a copolymer from a poly(aspartate) block grafted with comb-like poly(ethylene glycol) side chains containing a pH-sensitive imine bond PAsp(–N=C–PEG), a poly(L-cysteine block with a thiol group (PCys), and cation poly(aspartate) grafted with dimethyltryptamine (PAsp(DETA) Asp(–N=C–PEG)-PCys-PAsp(DETA). When exposed to acidic environments (pH 5.0), the nanoparticles prepared from this polymer cause cleavage of the imine bond, ultimately leading to the shedding of the PEG layer. This polymer was modified with lauric acid-targeting ligands to increase the delivery of the drug to THP-1 leukemic cells [79].

In another strategy, a pH-responsive acetalated dextran (Ac-Dex) polymer has been synthesized using dextran and 2-methoxypropene, which modifies the hydroxyl groups on the dextran into acetal groups [80]. This pH-sensitive polymer was water-insoluble and allowed for the fabrication of nanoparticles. These nanoparticles were encapsulated with a model fluorophore, fluorescein isothiocyanate-dextran (FITC-dextran), which showed a quick release at an acidic pH of 5.0 compared to the physiological pH of 7.4 [80]. This was attributed to the hydrolysis of the acetal group at acidic pH, leading to the conversion of original hydroxyl groups resulting in a water-soluble dextran and the release of the encapsulant readily [80].

The functional groups that are pH sensitive within acidic environments include imines, hydrazone, oxime, amides, acetals, and orthoesters (Table 3) [81]. Nanoparticles may be recruited to the tumor site passively through the enhanced permeability and retention (EPR) effect. Still, to better assist in the recruitment, pH-sensitive biomaterials may be used to change their ionization when exposed to the acidic tumor microenvironment [75]. This change in surface charge from negative to positive on the nanoparticle allows for enhanced uptake with the negatively charged tumor cell membrane. For example, Lou et al. prepared pH-triggered charge reversal, tumor microenvironment-responsive shell/core composite particles to deliver the anticancer therapeutic disulfiram (DSF).

In this study, DSF-loaded nanoparticles were prepared using a 12-hydroxystearic-poly(ethylene glycol) (PGlu-PEG) polymer and conjugated with TAT, a cell-penetrating peptide. When exposed to the tumor microenvironment, the carboxylic groups of PGlu-PEG got protonated, leading to the shedding of the PEG coat and subsequently releasing DSF. Furthermore, pegylated DSF-loaded nanoparticles showed the highest antitumor effect *in vivo* compared to free DSF solution, normal saline control, and DSF-loaded naked nanoparticles [86]. Figure 4 depicts the two methods of pH-sensitive release, including the proton sponge effect as well as this shedding of the PEG outercoat.

An exciting novel drug delivery strategy was reported by

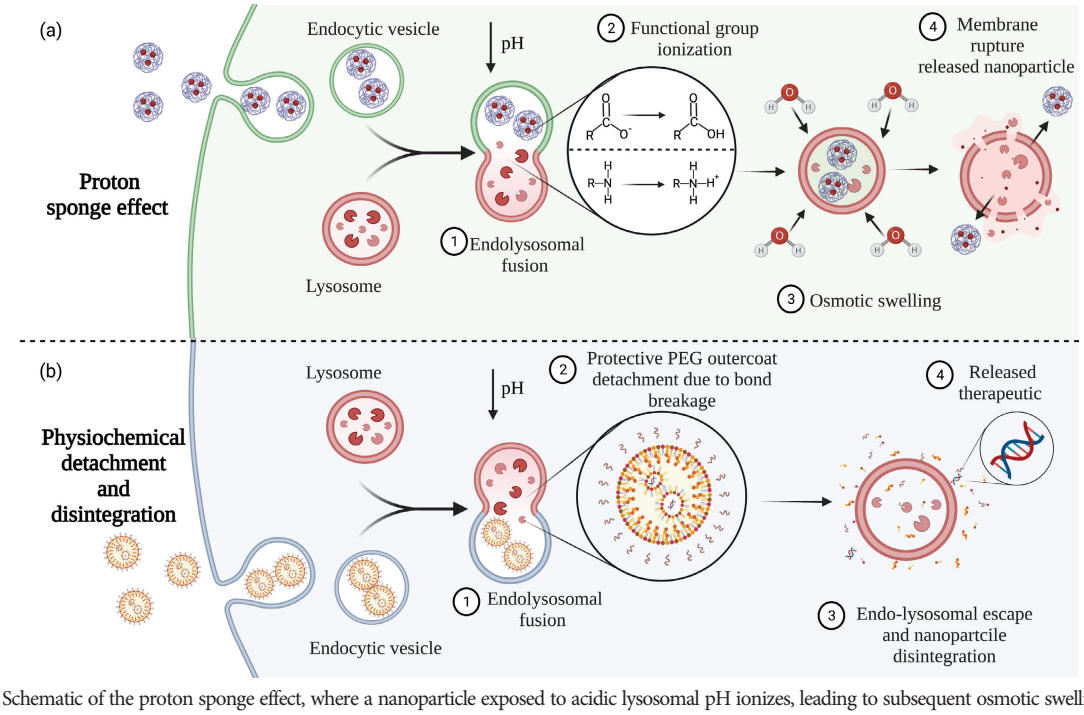
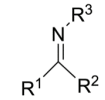
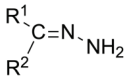
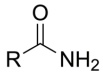
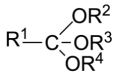


Figure 4 (a) Schematic of the proton sponge effect, where a nanoparticle exposed to acidic lysosomal pH ionizes, leading to subsequent osmotic swelling, causing the plasma membrane to rupture and nanoparticles to be released. (b) Additionally, the physicochemical detachment and disintegration which causes pH labile bonds to break, resulting in therapeutic escape. This image was created using Biorender.com.

Table 3 pH-responsive functional groups commonly used for intracellular delivery

Functional group		Description	References
Imines		Aldehyde hyaluronic acid-cistplatin (A-HA-CDDP) with imine bond between A-HA and CDDP	[82]
Hydrazone		Polymer-doxoxrubicicn (DOX) conjugate (PPC-Hyd-DOX-DA) with hydrazone bond between polymer and DOX	[77]
Oxime		Doxorubicin (DOX) encapsulated in poly(ethylene glycol) and oxmine tethered polycaprolactone (OPCL) micelles	[78]
Amides		Ovalbumin (OVA) peptide antigen conjugated to methoxypoly (ethylene glycol)-b-[polu(diisopropylamino)ethyl methacrylate) through a carbody-dimethylmaleic amide linker (CDM) (P-CDM-OVA)	[83]
Acetals		Pillar[5]arene (AC-PA[5] nanoparticles encapsulating paclitaxel and doxorubicin with a functionalized acetal group (AC-PA [5])	[84]
Orthoesters		Bromelain crossedlinked with an orthester monomer 4,4′-(oxybis(methylene)) bis (2-(2-(2-(oxiran-2-ylmethoxy ethoxy)-1,3-dioxolane) with encapsulated doxorubicin	[85]

Palanikumar et al. In this study, a hybrid nanoparticle synthesized from PLGA with bovine serum albumin (BSA) shell was utilized. This nanoparticle has been functionalized with an acidity-triggered rational membrane (ATRAM) peptide for specific tumor targeting. This ATRAM peptide relies on protonation of the glutamic acid, and upon exposure to the tumor microenvironment (pH 6.5), nanoparticles showed enhanced cellular internalization when compared to physiological pH conditions [87]. The ARTAM functionalized particles evaded rapid proteolysis and clearance *in vivo* and achieved highly efficient pH-dependent cellular uptake. The groups treated with the ATRAM-BSA-PLGA nanoparticles exhibited prolonged survival and antitumor efficacy compared to a control group receiving free doxorubicin [87].

While pH-responsive drug delivery systems are currently highly researched and have several advantages, like any delivery mechanism, there are also drawbacks. One of the significant limitations to this pH-responsive delivery is the heterogeneity of pH between various intracellular and extracellular spaces. This heterogeneity may result in the premature release of drug payloads. In addition, the proton sponge effect may be utilized as a pH-responsive delivery method, but it may not provide adequate therapeutic migration to the cytosol [88, 89].

4.2 Reduction oxidation potential

Another internal/endogenous stimulus impacting intracellular delivery is the reduction-oxidation potential (redox). The reduction-oxidation gradient between the extracellular and the intracellular environment often triggers the payload release. This redox gradient is due to the availability of GSH (Table 4), which is substantially lower in concentration extracellularly compared to intracellularly in the cytosol (Fig. 5) [90, 91]. Like pH responsiveness, redox-responsive nanomaterials reap similar benefits, including reduced systemic toxicity, precision drug release, and decreased premature drug degradation. Most nanoparticles sensitive to GSH typically contain disulfide bonds, which cleave into sulfhydryl groups in the presence of GSH, allowing the rupture of nanoparticles and subsequent release of the payload [92]

The disulfide cross-linking may appear anywhere within the components of a nanoparticle's core or shell. When the cleavage occurs between the inner and outer shell of the nanoparticle, the nanoparticle rapidly loses its integrity [92]. Recently, Kamenova et al. synthesized redox-responsive polymeric micelles from a block copolymer of poly(ε-caprolactone) (PCL), poly(acrylic acid) (PAA) and poly(ethylene oxide) (PEO) (PEO_{113}-*b*-PCL_{35}-*b*-PEO_{113} and PAA_{13}-*b*-PCL_{35}-*b*-PAA_{13}). The micelles were stabilized through crosslinking the PAA with cystamine dichloride, a disulfide crosslinking agent, and loaded with caffeic acid phenethyl ester (CAFÉ), a hydrophobic anti-cancer compound. Interestingly, these micelles showed no premature release of CAFÉ at pH 7.4 without a reducing agent compared to 100% release in 3 h when exposed to physiological pH in the presence of 10 mM dithiothreitol (DTT), a reducing agent [97]. Another study explored paclitaxel encapsulated dendrimer with tumor-targeting and cytosolic release abilities. Here, nanoparticles were responsive to intracellular glutathione and hydrogen peroxide (H_2O_2), cleaving the disulfide bonds. These nanoparticles demonstrated intratumoral accumulation of paclitaxel and quick release of paclitaxel when exposed to an oxidative environment [98]. In addition to utilizing disulfide bond cleavage through GSH, redox-sensitive biomaterials may also capitalize on ROS, which are abundant in multiple disease states [30]. These ROS-responsive systems have two essential mechanisms on which drug release may be based. The first is destabilization based on H_2O_2 breaking a carbon-heteroatom bond, and the second is changing the hydrophobicity of the nanoparticle to make it a more water-soluble [99]. Various reactive oxygen species include hydrogen peroxide, hydroxyl radicals, and superoxide anions, the bonds these ROS cleave include diselenides, arylboronic esters, thiolethers, aryloxalates, or ferrocene [30]. Some examples of reduction-oxidation sensitive biomaterials include methoxyl poly(ethylene glycol) (mPEG), poly(3-benzyloxycarbonyl-L-lysine) (PZLL), and aryl-boronate-modified dextran polymer (PDB-Dex) [30, 90, 100]. Poly(ethylene glycol)-*b*-poly(propylene sulfide) (PEG-*b*-PPS) block copolymer is one of the widely studied ROS-sensitive biomaterials to make morphologically diverse nanoparticles [101–103]. The hydrophobic PPS block of the copolymer

Table 4 Intracellular and extracellular GSH concentrations

Location	Concentration	References
Tumor tissue	~ 40 mM	[93–96]
Intracellular cytosol	1–10 mM	
Intracellular cytosol: brain	2–3 mM	
Extracellular plasma	4.5–20 μM	
Cerebrospinal fluid	5 μM	

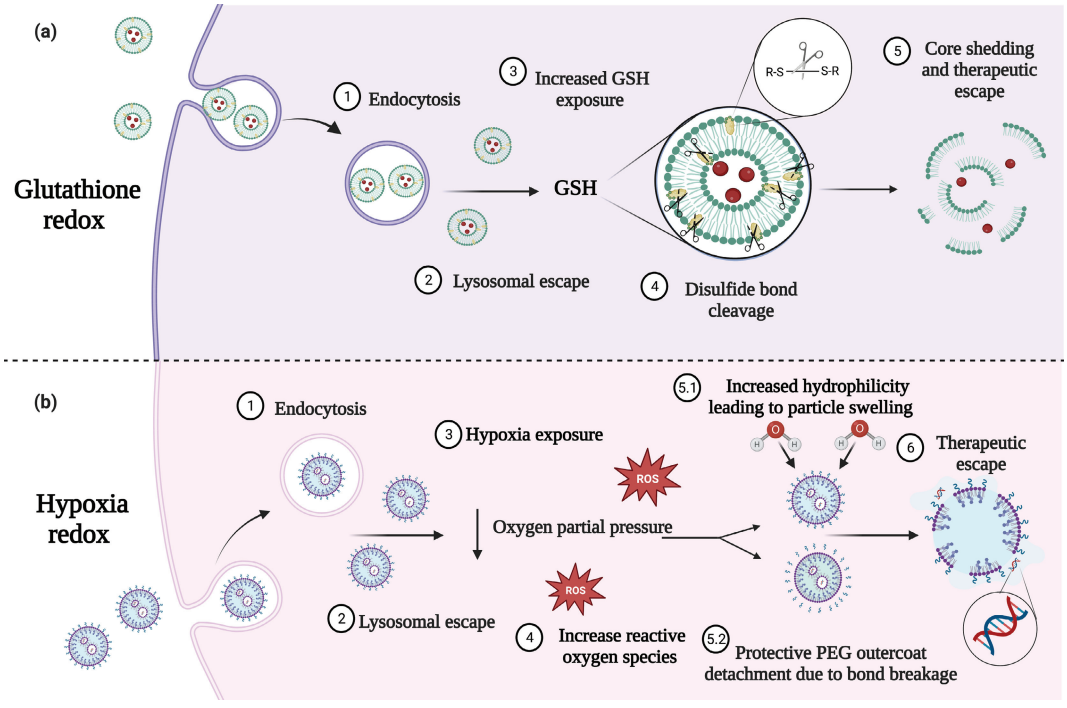


Figure 5 (a) Schematic of the GSH responsible oxidation showing the cleavage of disulfide bonds causing a core shedding effect and therapeutic escape, as well as (b) hypoxia-mediated redox conditions, cause endosomal escape and then bond cleavage. This image was created in BioRender.com

transforms into hydrophilic polypropylene sulfoxides or sulfones upon oxidation, causing the loss of nanostructure integrity and subsequent release of the payload. For example, Allen et al. encapsulated NF-κ inhibitor celastrol into PEG-*b*-PPS micelles to target inflammatory cell populations in the atherosclerosis region. Upon oxidation with H_2O_2, these nanoparticles triggered the 100% release of celastrol within 48 h, whereas the control group without H_2O_2 showed only 20% release even after 144 h [104]. Recently, Yi et al. developed a cytosolic delivery platform using a PEG-*b*-PPS copolymer conjugated to a cationic dendritic peptide [105]. This novel polymer formed monodisperse nanoparticles, which were non-toxic to immune cells and allowed the endosomal escape of the encapsulated payload. Strategies such as the intracellular transition of nanoparticle morphology have been reported using PEG-*b*-PPS copolymers for controlled delivery of payloads inside the cells. For instance, Bobbala et al. developed an interesting bicontinuous nanosphere platform that transitions into micelles upon intracellular oxidative conditions [101]. Furthermore, these nanoparticles showed enhanced retention of payloads in the cellular lysosomes and allowed the on-demand release of payloads into the cytosol via the morphological transitions [106]. In another study, Li et al. utilized tetra block copolymers composed of PEG-*b*-PPS, demonstrating the morphological transition of nanoparticles (cylindrical filomicelles to spherical micelles) when exposed to simulated oxidative intracellular environmental conditions [104].

Figure 5 depicts a subset of redox reactions with some enzymatic activity sensitive to hypoxia, which can target the low oxygen tumor microenvironments [30]. Hypoxia is highly prevalent among solid tumors, and with a drop in oxygen partial pressure, there is also an increase in ROS. However, a limitation is that early in the pathogenesis, when the tumor burden is not high, these hypoxia gradients are inadequate to induce these reactions [107].

Hypoxic tumor environments can cause an increase in drug resistance, which is why using hypoxia as a stimulus could be a promising approach. Like in other stimuli-responsive biomaterials, specific functional groups are sensitive to hypoxic situations, including quinones, nitroaromatic, and azobenzene derivatives that undergo a reduction in aminoaromatics [107, 108]. This reduction causes the nanoparticle to take on hydrophilic properties, resulting in the nanoparticle burst release of payloads. One example of the utilization of these hypoxia-sensitive bonds can be demonstrated in the study conducted by Yu et al. Self-assembled micelles were made of an amphipathic polymer consisting of chitosan (CS) and p-nitrobenzyl chloroformate (PNZ-Cl) with nitrobenzyl chloroformate as a hypoxia sensitive group (CS-PNZ-Cl). Here, nitroreductases present in the hypoxic tumor microenvironment caused nitrobenzene degradation and subsequent depolymerization of micelles to trigger drug release [109].

Interestingly, heterogeneity in enzyme expression leading to variable hypoxic levels in tumor cells was also addressed in this study. CS-PNZ-C1 micelles were loaded with a fluorophore, Nile red (NR), and release was examined in hypoxic and normoxic conditions to verify intracellular responsiveness. At the 4-h time, the hypoxic environment induced a 1.88-fold increase in the release of NR compared to the normoxic environment. Additionally, after loading with an anti-cancer drug, mitoxantrone, these nanoparticles were tested in a 4T1 breast tumor *in vivo* model, demonstrating tumor inhibition of 89.45% [109].

Additionally, the cleavage of hypoxia-sensitive bonds can be utilized to shed a nanoparticle coat of PEG to reduce steric hindrance and facilitate the cellular uptake [110]. It has been shown that by using this approach, solid tumors can be attacked in the core by targeting even the outer layers with tumor size undergoing a 200% reduction [111]. Hypoxia-sensitive formulations include biomaterial delivery vehicles such as liposomes, polymersomes, and polymeric micelles [108]. For instance, Mamnoon et al. prepared a polymersome formulation encapsulating doxorubicin, and this polymersome was synthesized using a PLA_{8500}-didiazobenzenebenzene-PEG_{2000} and PLA_{17000}-PEG_{2000}-Estradiol polymer. In this study, polymersomes were able to release only 30% of the doxorubicin under normoxic conditions, compared to a 90% release in a hypoxic environment (Fig. 5). It was proposed that the mechanism that facilitates this release is from the diazobenzene separating PLA and PEG due to cleavage by a reductase enzyme found in these hypoxic situations

[112]. The redox-responsive drug delivery systems have some disadvantages too. For example, heterogeneous GSH levels in various cell types could lead to premature degradation of the nanoparticles. Furthermore, a significant limitation is the ability to target cancerous cells while avoiding healthy cells precisely. This is currently being studied, and it is not uncommon to see dual-responsive drug delivery systems to try and mitigate these issues [113].

4.3 Enzymatic activity

Another group of stimuli-sensitive nanoparticles includes those that are responsive to intracellular enzymes. Dendrimers, polymeric micelles, and liposomes are delivery vehicles widely used in achieving enzyme responsiveness [108]. The mechanism by which enzymatic-responsive nanoparticles release their payload capitalizes on the presence of an enzyme, causing the nanoparticle to degrade. The enzymatic reactions can target multiple components of the nanoparticles, such as the cleavage of a responsive linker on the nanoparticles and the surface and core of the nanoparticles, as seen with other internal stimuli-responsive reactions. The application of enzyme-responsive carriers can be beneficial in cancer models due to the increased accumulation of certain enzymes at tumor sites.

Additional disease states that may capitalize on the upregulation enzymes for developing enzyme-responsive nanoparticles include inflammatory conditions such as inflammatory bowel disease and arthritis, as well as bacterial infections [114, 115]. There are various mechanisms that these stimuli-responsive carriers can undergo to release their payload; these include becoming ionically charged, cleavage of covalent bonds, and structural changes of the nanoparticle [116]. An example of enzyme-responsive bond cleavage was reported by Zheng et al. In this study, a polymeric nanoparticle synthesized using maleimide poly(ethylene glycol) (mPEG) conjugated via amino acid CAAN with lytic enzyme PTP-7 (mPEG-PTP-7) was utilized. CAAN is an asparagine endopeptidase and cysteine protease substrate (legumain). When CAAN is exposed to legumain, asparagine bonds undergo cleavage through hydrolysis, resulting in an enzyme-responsive system. This nanoparticle was encapsulated with paclitaxel prodrug, which works synergistically with PTP-7 to target tumor cells. Furthermore, paclitaxel is activated through a pH-responsive mechanism in the endolysosomal pathway [117].

Enzymatic responsive bond cleavage may occur through common enzymes such as matrix metalloproteinases (MMP), a hydrolase enzyme [110]. Based on their ability to break bonds, the most commonly utilized enzymes are hydrolases and oxidoreductases [118]. Mi and colleagues have compiled an extensive list of enzymes and their respective reactions [108]. Yildiz et al. synthesized a protease-stimulated nanoparticle for theranostic delivery to triple-negative breast cancer cells. The doxorubicin-loaded nanoparticle was made of poly(lactic-co-glycolic acid)-b-poly-l-lysine and poly(lactic acid)-b-poly(ethylene glycol). Poly-L-lysine (PLL) was covalently linked to Alexa Fluor 750 (AF750), a near-infrared (NIR) fluorescent molecule, to track the enzymatic cleavage. The PLL component of the nanoparticle is susceptible to degradation from protease enzymes such as cathepsin B and trypsin, which is required for the fluorescent signal to become quenched. When the particles were in the presence of trypsin, there was an immediate increase in fluorescence 2.6-fold, while in the absence of trypsin, there was no increase in the fluorescence [119]. In one study, a nanogel cross-linked to MMP-2 and MMP-9 substrates was studied to deliver auger electron-emitting nuclide (5-[^{125}I] Iodo-4"-thio-2"-deoxyuridine([^{125}I]ITdU)), which is radiotoxic when incorporated into DNA and serves as nano-irradiation. A diphtheria toxin receptor ligand (DTR) was utilized to transport the therapeutic across the blood-brain barrier to treat glioblastoma cells. The nanogel was synthesized using poly(ethylene glycol), as well as poly(ethylene oxide-co-propylene oxide) pre-polymers with acrylate end groups (Ac-sPEG). It was observed that the degree of degradation of the nanogels corresponded with the measured MMP-2 activity [120].

One of the limitations to the clinical applicability of this model is that the degree of enzyme activity will vary between patients. This is a significant limitation for all enzymatically responsive carrier systems, as the enzymatic activity may inherently be different at baseline for patients or may be pathologically changed due to the disease state being treated at various degrees.

5 External stimuli

External stimuli can also control the intracellular delivery of payloads from nanoparticles. Figure 6 depicts these stimuli, including ultrasound, light, electrical charge, and magnetic fields. These external stimuli can precisely enhance drug accumulation and retention in the desired area and promote the controlled release of the drug from the nanoparticles. Furthermore, external stimuli-responsive nanoparticles have been used for therapeutic delivery and as theranostics in cancer. Compared to internal stimuli-responsive nanoparticles, which depend on the physiological conditions it is exposed to, external stimuli-responsive nanoparticles have the advantage of being turned "on" or "off." The level of stimuli controls allows for unique dosing strategies that can be given to patients at regular intervals or continuously while providing a controlled and site-specific release of the medication. Here the mechanisms of the external stimuli-responsive nanoparticles are explained along with the recent advancements in the field.

5.1 Ultrasound

US technology is widely used clinically for diagnostic and imaging purposes and, more recently, has been studied for controlled site-specific drug delivery. US is composed of an acoustic wave with a frequency of more than 20 kHz; these waves have numerous physical properties such as attenuation, reflection, refraction, and amplification [121–123]. By controlling parameters such as frequency, intensity, and exposure time (continuous mode or discontinuous pulse mode), the drug delivery systems are tuned to achieve diagnostic and therapeutic effects in biological systems. A wide range of frequencies can be used for US. Low frequency (20–200 kHz) provides high tissue penetration with low resolution; contrary to that, high frequency (> 3 MHz) leads to low penetration but a high-resolution [122, 124–127]. The US transducer is responsible for converting these frequency waves into a pressurized mechanical wave responsible for localized density changes through compression and decompression, which can lead to an increased localized temperature [123, 128]. The mechanisms by which US is used for drug delivery can be categorized mainly as thermal, sonophoric, and chemical effects [123].

For thermal effects, US wave-targeted tissue absorbs the US energy in the form of heat, increasing the temperature in the surrounding area. The increase in temperature from 37 to 43 °C (usually called mild hyperthermia) induces blood vessel dilation, enhanced membrane permeability of vessel walls, and increased blood flow [129, 130]. Tumors are hypoxic and lack microvasculature, and this strategy is often used to design tumor-targeted drug delivery by increasing blood flow and decreasing hypoxia [131]. Additionally, this hyperthermic state results in increased tumor sensitivity to the chemotherapeutics, specifically

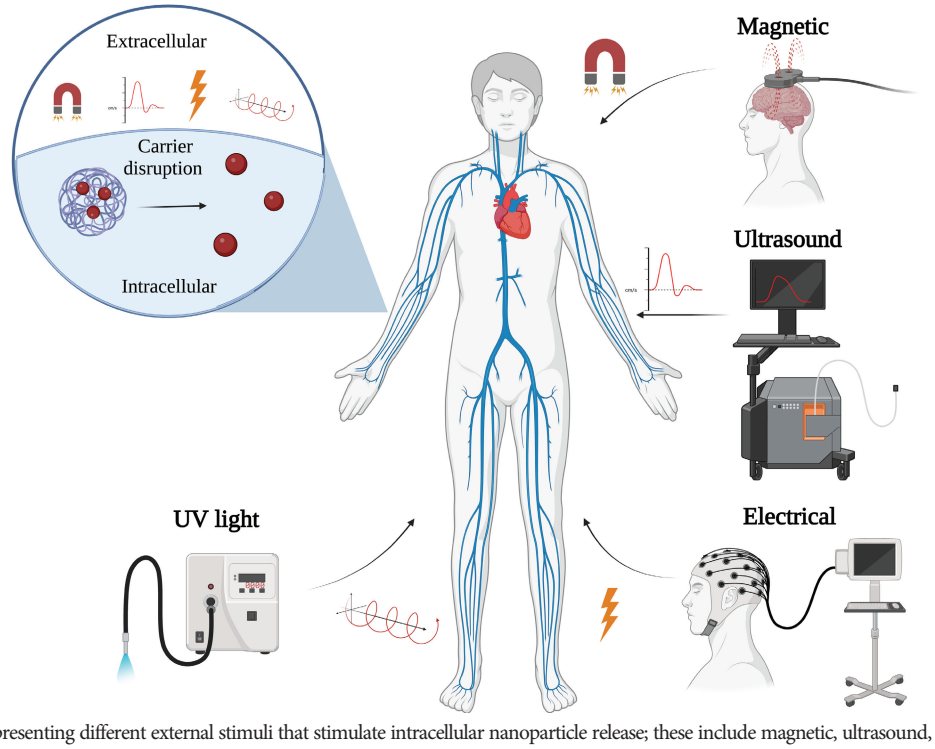


Figure 6 Schematic representing different external stimuli that stimulate intracellular nanoparticle release; these include magnetic, ultrasound, electrical, and UV light. This image was created in BioRender.com.

doxorubicin and cisplatin, which have been shown to display synergistic effects with hyperthermia [123, 131]. Thermodox®, a thermosensitive liposome containing DOX, is one of the most well-known formulations that employ this principle [132]. Thermodox® is a low-temperature liposome that is triggered to release its payload at 39–42 °C, in contrast to other traditional thermoresponsive systems that require higher temperatures to trigger release [131]. A drawback to hyperthermic temperature exposure is cellular killing and tissue necrosis, which can occur once temperatures reach 40 °C with an exponential reduction in survival with temperature with increasingly longer time points [123, 133]. Low-temperature sensitive liposomal (LTSL) formulations have the advantage of lower transition temperatures for drug release and a unique structural design.

LTSL formulations are designed with a faceted grain structure in the liposomal membrane; the acyl chains of the lipid membrane at the transition temperature result in boundary permeabilization, leading to structure disruption [128, 131]. This physical structure disruption is not the only mechanism in which hyperthermic reactions can affect drug delivery; there may also be an increase in the fluidity of the lipid components of the cell membranes, which subsequently increases the permeability, allowing for increased internalization of nanoparticles. However, since that is not focused on the inherent properties of the nanoparticle but rather the biological system, it will not be discussed further in this review [128]. An additional mechanism that utilizes the thermal properties of US is the use of near-infrared fluorescence (NIRF) labeled thermosensitive liposomes, which act in the presence of a high-intensity focused US (FUS) [134]. An example of this is topotecan (Hycamtin®), a chemotherapeutic agent loaded into the liposome, and NIRF imaging was used to locate the liposome accumulation in the tumor.

Interestingly, after the intravenous administration of these liposomes, FUS enhanced the cellular uptake of liposomes by inducing hyperthermia that was followed by a rapid release of topotecan inside the cells [134]. An advantage of this focused method of using US is that the focus can be localized to just a few cubic millimeters, which helps with accuracy in tissue targeting and precise nanoparticle disruption [128]. A disadvantage to the thermal responsive nanoparticles triggered through US is the limited materials that can be utilized. To be adequately responsive to thermal shift, nanoparticles must be comprised of specialized phospholipids or polymers to undergo destabilization under hyperthermic environments. Furthermore, these nanoparticles should also maintain stability at normal body temperature and allow payload release with sharp sensitivity upon a slight temperature increase [128].

Another mechanism in which US can trigger specific drug release is through shear sonoporation disruption, resulting in further membrane disruption and facilitating a passive nanoparticle uptake [128]. Inertial cavitation of gas microbubbles produces liquid jetting and shockwaves upon collapsing; this mechanical effect enhances the drug delivery outcome [121, 135–137]. Nested nanobubbles have shown US-triggered drug release; the nanobubbles were prepared using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]) (DPSE-PEG2000). Nested nanobubbles were created by mixing a combination of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), cholesterol, and DSPE-PEG2000. Calcein was loaded as a model drug, and a drug release profile was observed under the influence of the US. It was reported that the nanobubble population contains gaseous and liquid phases, which undergo a low-pressure phase change under the influence of the US. The drug was released because of the synergistic effects of mechanical and thermal stimuli generated by the US treatment [138].

US-mediated drug delivery and US contrast agents such as microbubbles gained much attention in the drug delivery field. The smaller size of the nanobubbles not only takes advantage of EPR effects in the cancer environment but also provides its contrast and drug delivery benefits [139]. In the presence of US waves, size oscillation occurs in the micro or nano-sized bubbles [123, 140].

Changing the parameters under which US is applied may result in two types of cavitation: stable and inertial [128]. Stable cavitation is achieved by using sustained US frequencies to match the frequency of the oscillating bubbles, leading to equilibrium;

this is advantageous when a shear force is desired as it achieves a particle disruption [123]. In contrast, negative pressures cause rapid expansion and subsequent collapse of microbubbles, which is inertial cavitation, also referred to as ultrasound-targeted microbubble destruction drug delivery [123]. Inertial cavitation is especially advantageous as a drug delivery mechanism as it allows a decreased pressure threshold to achieve results without damaging surrounding tissues [123, 141].

The chemical reactions which occur after US are referred to as sonochemistry and occur at a cellular level [123]. Most commonly, this is due to ROS, which can result in cellular necrosis and apoptosis [123]. The generation of ROS has major disadvantages; however, producing increased levels of ROS can result in oxidative damage to healthy cells and induce carcinogenesis through DNA fragmentation, autoimmune diseases, neurodegeneration, and ischemic injuries [128, 142]. The other biochemical reactions include increased intracellular calcium, which is vital after the sonoporation [128]. The calcium transients impact endocytosis pathways and the self-sealing membrane [143]. Additionally, when treated with a US intensity higher than 2.1 W/cm^2, hyperpolarization happens instantaneously after exposure and then depolarizes after 180 min [144].

There are some challenges when using US to enhance drug delivery. One of the significant logistical limitations is the ability to configure and calibrate the instruments to allow for specific parameter control, which will vary for each intended use. Specifically, the configurations can dramatically alter the applications of the acoustic beam and transducers. So, each condition must be systematically calibrated and validated to ensure accuracy [128]. This time-consuming and expensive process may limit this method's applicability. Additionally, there are challenges regarding ROS damage to healthy cells while maximizing the intensity to adequately disrupt the cellular membrane to allow for increased nanoparticle internalization.

5.2 Light

Light as a stimulus for intracellular drug release is considered a flexible and spatiotemporal stimulus [145]. Light-sensitive moieties such as nitrobenzyl group, perylene, cardiogreen, and riboflavin chromophores have been incorporated into nanoparticles for stimulated release [145]. Light-responsive nanoparticles must rely on the inherent properties of the particle as well as the properties of light that trigger the response. The main properties of light that impact drug delivery include wavelength, power, and penetration [146]. Light is already used clinically in photodynamic therapy and photocoagulation; while it is easily tunable for spatiotemporal applications, some toxicities are associated with its use [146]. For any clinical significance to occur from photosensitive drug delivery, adequate tissue penetration must be impacted by the selected absorption and scattering. NIR light penetrates tissue well at wavelengths of 650–900 nm and is desirable for drug delivery stimulation; NIR, however, is not the only form of light utilized [146]. Ultraviolet (UV, 200–400 nm) and visible light (400–700 nm) have also been studied. However, the disadvantage is that the resulting penetration depth is significantly less than NIR [146, 147]. Light-responsive drug delivery systems are being developed based on photochemical, photothermal, and photoisomerization strategies. Toxicity is associated with photochemical and photothermal reactions [146]. Most commonly, the carriers that utilize these mechanisms include micelles, liposomes, and inorganic nanoparticles [148].

Photochemical-based drug delivery systems use light irradiation to trigger covalent bond cleavage of the material to release drugs. For example, the most commonly used ortho-nitro-benzyl (o-nitrobenzyl) group in photolabile delivery systems generates o-nitrosobenzaldehyde and free carboxylic acid after exposure to a UV light [149]. One of the most used photolabile materials, such as ortho-nitro-benzyl derived materials, undergoes a conversion reaction to form an acinitro intermediate. Acinitro intermediate generates a nitrosophenyl carbonyl compound by cleaving the carbon-heteroatom bond at the benzylic position [149, 150].

A disadvantage to photochemical reactions is that they are limited to UV and visible light spectra, which require higher energies, resulting in poorer tissue penetration while also having an increased risk of toxicity and tissue injury [146]. Another example of a photochemical reaction is photosensitization; this acts in a similar mechanism to US-triggered biochemical processes by producing ROS, which may result in further tissue injury, DNA damage, and ultimately cell death [146]. Other photochemical-based reactions, in addition to cleavages, include rearrangements that change the hydrophilic-lipophilic balance [146]. The photochemical rearrangement reaction was demonstrated with micelles synthesized from hydrophilic PEG conjugated to a hydrophobic 2-diazo-1,2-naphthoquinone (DNQ), with DNQ being the light-sensitive moiety [151]. When DNQ was exposed to UV or visible light, it underwent a Wolff rearrangement, forming a hydrophilic 3-indene-carboxylic acid, which caused the rupture of micelles and release of the encapsulated cargo [151].

In another study, an exciting photothermal strategy was used to release drugs from nanoparticles made up of organic phase change materials (PCMs) such as lauric acid (m.p. = 44 °C) and stearic acid (m.p. = 69 °C). A eutectic mixture of the two fatty acids at a weight ratio of 4:1 was used for the formulation and possessed a melting point of 39 °C. These nanoparticles were loaded with a near-infrared dye, IR780 iodide (IR780), and a model anticancer drug, DOX, to study the cytosolic release in A549 cells. Upon near-infrared radiation, the temperature reached beyond the eutectic point, resulting in the melting of the nanoparticle and subsequent intracellular drug release into the cytoplasm [135]. In addition to these materials, compounds like cinnamylidene acetate, nitrocinnamate, and anthracene have been widely studied because of their light-induced reversible dimerization properties [150, 152, 153].

On the other hand, photoisomerization-based drug delivery systems depend on the UV or visible light-mediated reversible change of the material properties. The most widely studied compound for these delivery systems is azobenzene. They contain two phenyl groups that can change from trans to cis conformation upon UV light irradiation (300 to 400 nm). This conformational feature also serves as a turn-on and turn-off switch for drug delivery systems to control cargo release [154]. Photo-oxidation mediated cytosolic delivery of encapsulated payloads has been studied using polymeric bicontinuous nanospheres (BCNs) prepared from PEG-*b*-PPS. As mentioned earlier, the hydrophobic PPS block of the polymer is prone to oxidation and increases the hydrophilicity of the copolymer, which helps in disassembling the PEG-*b*-PPS nanostructure. BCNs encapsulated with a photosensitizer, pheophorbide A, generated ROS species upon light irradiation, which not only oxidized the PPS block but also mediated the intracellular release of the drug molecules from the lysosomes to the cytoplasm [106]. The advantages of light-responsive systems are that they tend to be highly focused, even more so than the US, and operate with a wide range of absorbances, including NIR, UV, and visible spectra. However, many challenges need to be addressed, including the low penetration ability and the tissue damage that can occur with increasing thermal response [147, 148, 155].

5.3 Magnetic

The magnetic responsive nanocarrier's tropism to the magnetic field and the ability to generate hyperthermic conditions under alternating magnetic fields (AFM) make it an attractive nano-drug delivery platform. Based on the orientation of the influence of a magnetic field, any magnetism of a particle can be classified as diamagnetism, paramagnetism, ferromagnetism, ferrimagnetism, or anti-ferromagnetism [156]. The superparamagnetic behavior of nanoparticles is considered ideal for many biomedical applications [156–158]. Some of the most common magnetic nanoparticles include iron oxide, graphene hybrids, and zinc ferrite [159–161]. Notably, many magnetic nanoparticles are either stabilized using biocompatible polymers or encapsulated in lipid or polymeric nanoparticles to overcome stability issues and enhance cellular delivery. For instance, Thirunavukkarasu et al. fabricated superparamagnetic iron oxide (Fe_3O_4, SPIONs) nanoparticles for theranostic applications. SPIONs and anticancer drug DOX were co-loaded in temperature-responsive poly PLGA (T_g = 42–45 °C) nanoparticles. In the presence of an external AMF, SPIONS induced a local hyperthermia environment, which not only stimulated the release of the doxorubicin but also benefited the cancer treatment when tested in a mouse tumor model [162]. Similarly, Zhong et al. prepared folic acid-functionalized reduction-responsive magnetic chitosan nanocapsules (FA-RMCNCs) using folic acid-functionalized thiolated chitosan and thiolated Fe_3O_4 nanoparticles. Nanocapsules exhibited greater magnetic responsive ability, and intracellular cleavage of disulfide bonds triggered the release of coumarin 6 dye, which suggests the dual responsive behavior of these nanocapsules [145]. In another study, magnetic and temperature-sensitive solid lipid particles (SLPs) were developed using oleic acid-coated iron oxide (IO-OA) nanoparticles with 1-tetradecanol and poly(ethylene oxide)-block-poly(ε-caprolactone). A dose-dependent cytotoxic effect was observed with magnetic SLPs in JURKAT cells compared to non-toxic non-magnetic SLPs [163].

Magnetic field-responsive carriers are typically safer, exhibiting less tissue damage with high levels of precision, and the tissues only remain affected while the magnetic field is activated; this can be compared to some of the biochemical reactions discussed with other external stimuli, which can be irreversible [148]. Magnetic fields are essentially indiscernible to tissues when they pass through, for they are neither disordered nor absorbed.

5.4 Electric

Materials such as polypyrrole (PPY), ferrocene, and carbon nanotubes exhibit electrically conductive properties and are used in drug delivery systems responsive to electrical stimuli. For drug delivery applications, approximately 1 V of weak pulses are commonly used [118]. The delocalization of μ electrons and the μ-conjugated backbone in the polymeric materials results in electrical conductivity in the nanoformulations. Anionic dopants are used to stabilize the delocalized electrons, which also oxidize the polymer and create a continuous conduction band. Under a weak pulse, the electro-responsive polymer releases the cargo due to the electrostatic repulsion [164, 165].

From a drug delivery standpoint, most methods related to electro-responsive formulations include the fabrication of electro-responsive films and dispersed electro-responsive formulations. PPY can be electropolymerized easily on any conductive surface. As a result, it is one of the most commonly used intrinsically conducting polymers in the biomedical sciences as films. However, the use of these films is highly limited for intracellular drug delivery applications. Recent advancements include colloidal systems that are responsive to the electrochemical gradient. These systems can be easily injected without any need for implantation. Meng et al., for example, reported the creation of electro-responsive micelles composed of Pluronic F127 and d-tocopherol polyethylene glycol succinate (TPGS) conjugated with the electro-sensitive group ferrocene (Fc) (TPGS-Fc). Oxidation of the reduced Fc in the presence of electrical stimuli was able to release the drug via disruption of the micelle structure. When encapsulated with model drugs, rhodamine 123- and DiR, these micelles showed controlled drug release *in vivo,* corresponding to the electrical stimuli applied [166].

6 Conclusions

Drug delivery has made tremendous advances in the last decade. Nanoparticle-based therapies are increasingly common, with intracellular delivery highly impactful and sought after, especially for oncological indications. As nanoparticles continue to advance and make it to the market for clinical use, they are traditionally composed of soft organic materials with flexible and elastic properties to traverse through the body. In non-targeted approaches, nanoparticles are internalized through general mechanisms, including clathrin-dependent and independent endocytic pathways. After internalization, particles are exposed to various subcellular pathways in which nanoparticles can interact and release therapeutics to organelles. These organelles affected are most commonly the nucleus, the lysosomes, mitochondria, Golgi, and the endoplasmic reticulum. Nanoparticles may also be internalized through surface modifications to functionalize particle surfaces with cell-specific targeting ligands. Still, another step that needs further research includes the ability to functionalize particles to precisely target subcellular components. While not specifically a targeting mechanism, nanoparticles can be manipulated and functionalized to achieve drug therapy with a more controlled and specific therapeutic release. One way that these materials may be utilized is in a stimuli-responsive drug carrier system. Stimulus responsiveness can be achieved due to internal or external stimuli, with internal stimuli relating to the endogenous properties of different physiological spaces.

In contrast, external must be exposed to an exogenously applied force. Most intracellular delivery systems often show imprecise release profiles after *in vivo* administration, which limits their usage, which can be addressed through these stimuli-responsive nanoparticles to release drugs inside the cells. Internal stimuli which may cause this triggered release include pH, reduction-oxidation potential, and enzymatic interaction. Internal stimuli have one significant advantage directly over external stimuli; internal stimuli-responsive nanoparticles are better suited when precise intracellular release is desired. The release mechanism depends on a specifically targeted cell environment, which means therapeutic drugs are retained inside nanoparticles until they reach that environment. This is ideal for drug targets that are not static. However, one major issue with internal stimuli-responsive systems is the lack of control over the release of the drug at the site of action. For example, an encapsulated drug may be released rapidly from the nanoparticles once an internal target stimulus activates the nanoparticle rupture. This phenomenon may not be desired in every disease condition as sustained therapeutic benefits could be compromised, requiring multiple administrations of drug doses.

As opposed to internal stimuli, external stimuli are not as widely utilized for the intracellular release of drugs as previously thought; however, this strategy relies on release profiles stimulated by more readily tunable means of control. The methods that are used to externally trigger the therapeutic release of therapeutics encapsulated within nanoparticles include ultrasound, light, magnetic and electrical forces. A disadvantage to externally stimulated drug release is that not only do the biomaterials need to

have stimulable properties but there also needs to be an encapsulated external agent. This external agent is what is being activated to trigger the drug release mechanism and is often in addition to other encapsulated therapeutic agents. Of note, if a trigger needs to reach endogenous organelles of cells in the circulation or deeply targeted tissue, using external stimuli is a difficult strategy. Since the release profile depends on externally controlled factors, this technique is better suited when time-sensitive release profiles are desired rather than precise location release. Furthermore, external stimuli also allow "on" and "off" mechanisms, which help control the drug release as required.

Additional challenges which warrant further investigation include the stability of nanoparticle formulations. Stability is essential for stimulatory responsive nanoparticles because any factors related to storage conditions such as light, oxidation, and temperature could play a key role in stimulating drug release in a storage vial if a biomaterial is required to utilize any of these specific properties as a trigger for intracellular release. There are also challenges with the stimuli themselves due to the heterogeneous nature of cellular environments, and something worthy of future studies to address. Overall, stimulatory-responsive nanoparticles advance drug release strategies by allowing for increased precision in drug release mechanisms. Many soft nanoparticles studied for stimuli-responsive studies are biocompatible, biodegradable, and non-cytotoxic. However, the complex chemistries in synthesizing these biomaterials must be simplified for scalability and translation purposes.

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