Design and Evaluation of Multi-Stimuli-Responsive Nanoparticles for Targeted Drug Delivery in Chronic Diseases

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Abstract

The key reasons for disease and death global, cancer, diabetes mellitus, cardiovascular, chronic inflammation, neurodegenerative and chronic kidney, liver, lung disorders are the top of chronic diseases, and treatments have faced several limitations as the reasons are based on multifactorial pathology, biological barrier, intrapatient disease heterogeneity, poor accumulation of drugs in the lesion sites, general toxicity as well as the absent of drug-release mechanisms responding to changing microenvironment of the lesion site. The innovative smart drug delivery platforms of multi-stimuli-responsive nanoparticles have emerged in the field of drug delivery for combining two or more endogenous and/or exogenous stimuli to realize targeted release, spatially controlled drug release, and temporally controlled release. The endogenous stimuli, including decreased pH, redox gradient, ROS, enzyme, hypoxia, glucose, and ATP, can be combined with external stimuli, including temperature, light, magnetic field, and ultrasound, to trigger targeted delivery of nanoparticles and enhance the specificity of drug release. In the present work, the principles, classification, design principles, material selections, and engineering strategies for the development of multi-stimuli-responsive nanoparticles, including the sequential stimulation and co-stimulation mechanisms, the cascaded responsive nanocarriers, and the charge-reversal and size-transformable nanocarriers were critically presented. Different nanoparticle systems, including polymer nanoparticles, micelles, nanogels, liposomes, lipid–polymer hybrid nanoparticles, mesoporous silica nanoparticles, magnetic nanoparticles, metal–organic frameworks, and biomimetic systems, were highlighted. Stimuli-responsive building units and self-assembly techniques for them were also reviewed. The disease applications of several models, including cancer, diabetes, cardiovascular diseases, inflammatory diseases, neurological disorders, kidney diseases, hepatic diseases, and lung diseases, were evaluated and debated. Despite encouraging developments, challenges remain for clinical translation, such as biological variability, uncertain trigger intensity, off-target delivery, premature leakage of drugs, formation of a protein corona, and immune rejection; difficulties in formulation and scale-up production, reproducibility, stability, uncertainty in toxicity, and changing regulatory guidelines. The responsive nanoparticles, in the light of developing new, complex designs, may eventually transition from “smart” but static nanocarriers to “intelligent and dynamic” nanotherapeutics, which must also, in parallel, consider balancing the complex responses with ease of preparation, safety and reliability as well as clinical potential.

Keywords: Nanoparticles; targeted drug delivery; stimuli; precision therapy; personalized medicine.

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Introduction

Today's controlled release drug products solely control the rate at which the drugs are delivered. The delivery of these drugs is unresponsive to triggers at the right time of treatment. To overcome such a problem, it is necessary to provide high dose and /or repeated doses of the drugs. Cardiovascular Diseases, cancers, diabetes, and chronic diseases of the respiratory tract account for significant disability and premature deaths worldwide, and with ageing populations, increasing urbanisation, lifestyles with low physical activity, poor eating habits, environmental exposures, and the presence of metabolic syndrome[1]. They can take many years and/or the entire life of a patient to heal, so in contrast to the acute types, it is very important to maintain medical therapies for a long time. Those long-term therapeutic medical approaches are difficult due to: higher drug doses, poor adherence, accumulating toxicity, development of resistance, varied response to treatment, individual sensitivity, and accumulation of side effects. The complex nature of chronic disease pathologies with alteration of cell

metabolism, inflammation, tissue and blood vessel Architecture, immune activity, and cell and tissue microenvironment dynamics has paved the way for the development of more intelligent drug-delivery systems which may enhance delivery selectively to a pathological area[2].

Standard drug formulation procedures have a very limited degree of control over both time and space for the drug release from the carrier after administration. Upon drug delivery, the system travels throughout the body in circulation and interacts not only with pathological tissues but with other body tissues also, reducing local concentrations of drug at a target area and resulting in non-specific drug delivery and thus toxic side effects. The majority of these drugs exhibit very poor solubility in water and poor permeability of cellular and biological barriers; hence are degraded in the body before reaching the site of administration or are rapidly eliminated from the systemic circulation by metabolism in the liver[3]. Many of these drugs also have a very short half-life, low volume of distribution, high protein binding, and poor BioAvailability (due to extensive metabolism by the liver); thus, to reach sufficient drug concentrations, often need to administer high or frequently repeated doses.

The existing controlled-release drug formulations only control drug release, with no responsiveness to the biological cues at the time of need for treatment. Many factors limit successful delivery of these Drugs to pathological cells: The barrier between administrative site and cellular and biological tissues. These barriers include the gastrointestinal degradation of some Drugs; breakdown of the drug due to low pH; mucus coating; disruption of epithelial tight junctions; Vascular endothelium; and metabolism in the liver and elimination in the renal system. Additionally, the mononuclear phagocyte system (MPS) in the body will clear the Nanocarriers from circulation. Once in the tissue, they need to penetrate dense extracellular Matrices and abnormal interstitial pressure before reaching target cells; intracellular degradation or entrapment in endosomes or lysosomes would occur to therapeutic Drugs[4].

Several chronic diseases possess extremely challenging Barriers. For Cancer Diseases, the tissues have irregular vascular Architecture, interstitial fluid pressure, a dense matrix of fibers, and acidic and hypoxic regions. Chronic inflammationand AutoimmuneDiseases, the blood vessel permeability may fluctuate, and the cell immune activities vary. Furthermore, the surface of nanoparticles gets coated by plasma proteins, and a biomolecular corona forms on its surface that alters the nanoparticle surface characteristics, circulation behavior, targeting efficiency, and cellular uptake. Therefore, successful delivery is not just about encapsulating Drugs but delivering and triggering drug release within targeted sites, passing successively through a range of physiological and pathophysiological Barriers[5].

To overcome these limitations of traditional systems, a targeted approach for drug delivery has been established using nanocarriers. Such benefits have been achieved, including enhanced aqueous Solubility of hydrophobic drugs, improved resistance to the rapid degradation of labile agents, prolongation of the time of circulation in the systemic body, change in physiological activity, and enhanced cell delivery and accumulation of target agents in pathological tissue. Passive targeting utilized the pathological features of target sites, such as leaky blood vessels or recruited immune cells for inflammatory sites. In the active targeting system, ligands such as antibodies, peptides, aptamers, carbohydrates, or even small molecules that interact with specific cellular target-associated molecules are decorated on the nanocarrier's surface[5].

However, conventional targeted nanocarriers also face challenges in heterogeneity of expression on disease cells, in heterogeneous vascular permeability, non-specific cell uptake, plasma protein adsorption, and control over drug release once reached thetarget sites. As it is known that when a targeted Nanocarrier arrives at the targeted area, the drug could be released too early, or the tissue can't be penetrated easily, or the cell traps the nanocarrier in the endosome, or the clearance time of the drug becomes shorter. Hence, a precisecontrol in drug release at specific sites based on biological or pathological context is required. As such, stimuli-responsive nanocarrierswere inventedto tackle the problem, since these carriers are designed to release their payload upon exposure to external or endogenous stimuli. The triggers may include intrinsic(pH, ROS, redox potentials, glutathione concentrations, specific disease-associated enzymes, low Oxygen level (hypoxia), glucose levels, inflammatory Mediators) and external triggers(temperature, light, sound (US), magnetism, and electrical fields). These responses may involve bond Cleavage, CarrierSwelling, carrier Degradation, and changes in permeability of cell-like membranes of Nanocarriers, Charge reversal, structural change of nanocarriers, etc. For controlled Drug Release. Single-stimuli-responsive systems represent a significant advancement over traditional formulations[6].

They show relative stability in physiological conditions but can respond to an environmental condition associated with disease states, thereby triggering drug release and facilitating tissue repair. However, due to the heterogeneity and complexity of pathological tissues in diseases such as tumors and chronic inflammations/autoimmune diseases, single-stimulus-triggered systems might not be enough for

achieving desirable treatment effects, as only one specific abnormality signal might be weak or fluctuate. The microenvironment of chronic diseases is often multifaceted, geographically diverse, and temporally changing. For example, tumors are often accompanied by an acidic tumor Microenvironment (TME), increased intracellular redox conditions, high ROS, overexpressed enzymes, and Hypoxia. Diabetic disease complications are also accompanied by hyperglycaemia, oxidative stress, inflammation, low vascularity, and some pH abnormalities in tissue Microenvironments. Chronic inflammations/autoimmunediseases also cause high generation of ROS, acidic local microdomains, activity of proteases, and elevated levels of inflammatory mediators. Furthermore, different signaling levels may vary from one patient to another and at different disease stages. So single- stimulus triggers may result in insufficient triggering and activation, premature release of the drug, and poor discrimination between pathological and normal tissue[7].

To address these deficiencies, a multiple-stimuli-responsive nanocarrier has been developed which can be simultaneously activated by multiple endogenous/exogenous triggers (multi-stimulus responsive systems) in the disease microenvironment. E.g., pH-ROS dual, pH-redox dual, pH-enzyme dual, ROS-enzyme dual, glucose-pH dual, hypoxia-ROS dual, Temperature-pH dual, magnetic field-pH dual, etc. Multi-responsive Nanocarriers can trigger their Drug release by either sequential action, the synergistic effect of the triggers, or using specific logic (e.g., AND logic system, OR logic system). Sequential responsive mechanisms can enhance drug delivery in some cases by first carrying out some physicochemical changes that result in desheilding of the drugs, surface charge changes, or size alteration that lead to increased penetration into tissues, or improved Cellular uptake. Then, an intracellular stimulus will trigger drug release. AND logic system requires the simultaneous triggering by at least two stimuli for successful release of drugs, so the targeting ability is significantly increased.ORlogic systemis a good choice for diverse diseased environments, since either of several stimuli could trigger the release of drugs[8].

The reason for designing multiple responsive Nanocarriers is therefore from the biological process of chronic diseases. These nanocarriers function as a controllable system to respond to different signals present in pathological tissues, integrate different external and internal signals to execute a triggered and controllable delivery process, which is advantageous in delivering drugs with reduced drug leakage in non-specific locations, efficient accumulation in pathological tissue, and improved Tissue penetrability and intracellular delivery; the sequential penetration barriers overcome and reduce the overall toxicity of drugs (Figure 1). They can also provide an effective delivery platform for combination therapies, delivering multiple drugs or therapeutic agents with the same or different temporal release profiles. Multi-responsive nanocarriers, a more logical platform, are more promising to implement a new concept of 'precise nanomedicine'. Although challenges have been overcome to some extent in synthesis complexity, reproducibility, scaling-up processes, and the safety evaluation of the smart nanocarriers to apply to further clinical translations[9].

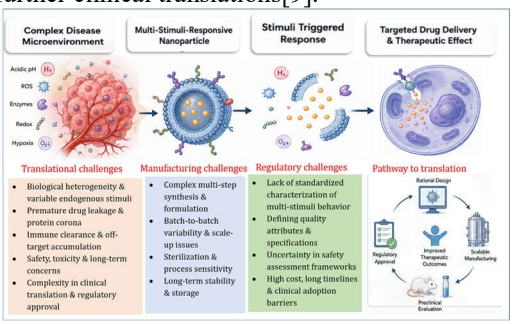

Fig.1. Summary of multi-stimuli-responsive nanoparticle designs with disease-specific microenvironmental triggering, stimulus-responsive drug release mechanism, target therapeutic effect, and major translational, manufacturing, and regulatory barriers to clinical translation

Classification of Stimuli-Responsive Nanoparticles

Internal triggers refer to the stimulations in a biochemistry change related with disease (i.e., acidic pH, redox, reactive oxygen species (ROS), enzyme, hypoxia, glucose concentration, and ATP concentration); on the other hand, external stimulus means stimulations applied from the external like UV, Visible, NIR, temperature, magnetism, and ultrasound. Here, stimuli are defined as the significant factors that cause changes in the physical/chemical properties or structure of the nanocarrier. This can involve modifying certain elements within a nanocarrier structure, such as cleavable chemical bonds, ionizable functional groups, responsive polymers, lipids, proteins, and inorganic materials. When a stimuli-sensitive system encounters its predetermined stimuli (e.g., acidic pH, enzyme, light, temperature, etc.), it undergoes a physical/chemical change (e.g., swelling, degradation, bond breakage, flip-flop behavior, phase transition, etc.) or it disintegrates, leading to drug release in a spatially- and/or temporally-controlled manner. Depending on the number of stimuli involved, the responsive nanocarriers are classified as single-stimuli-

responsive and multi- stimuli-responsive nanoparticles. The single-stimuli-responsive nanocarriers are responsive to a single stimulus and offer an advantage in simplicity of formulation, characterization, and production, but may have a disadvantage due to the complexity and variability of pathological microenvironments. The multi-stimuli-responsive nanocarriers employ more than two stimuli to improve selectivity, adaptability, and precision of drug release in combination through various schemes: sequential, simultaneous, AND-gate, or OR-gate manner. The stimuli are further divided into two types: internal and external triggers. Internal triggers correspond to the stimuli in a disease-related biochemical change (e.g., acid pH, redox, reactive oxygen species (ROS), enzyme, hypoxia, glucose concentration, and ATP concentration), while external stimuli are externally applied, such as UV, visible and NIR lights, temperature, magnetism, and ultrasound (Figure 2). Thus, integration of diverse internal and external stimuli in the multi-stimuli-responsive nanoparticle designs represents an excellent tool for the programmable and disease-directed delivery of drugs[9].

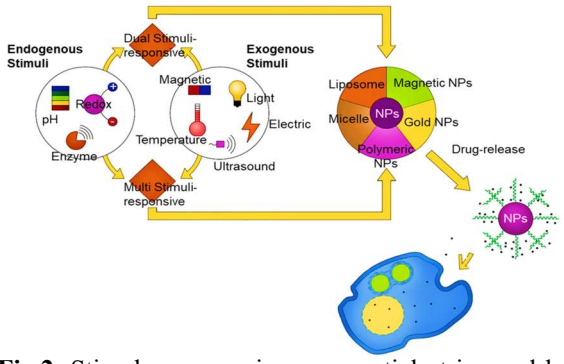

Fig.2. Stimulus-responsive nanoparticle triggered by exogenous stimuli stimulus-responsive system stimuli

Design Principles and Engineering Strategies for Multi-Stimuli-Responsive Nanoparticles

The surface can also be functionalised further with the addition of a peptide, protein, antibody, aptamer, carbohydrate, or small molecule to the appropriate targeting receptor for the disease of interest. The choice of stimulus should be based on: intensity of stimulation; area of stimulation; space/time of coverage; link of stimulation parameters to the disease pathogenesis; discrimination of disease tissue vs healthy tissue. Switching properties on-demand: The size, charge, and shape of the nanoparticles could be manipulated upon need. Specific probes used to target: e.g., Stimuli-responsive targeted probes. - Charge-reverse particles; these particles are either neutral or negative in the bloodstream, while they are positively charged in diseased tissues, which may facilitate cellular binding and internalization. - Size-switchable nanoparticles; sizes can be changed specifically to obtain long circulation time and deep tissue penetration[9].

In Simultaneous responses, the nanoparticles respond to the applied stimuli concurrently, while in sequential designs, structural transitions prompted by one trigger expose responsiveness to a second. Dual- or triple-responsive nanoparticles comprise combinations of two or three mutually beneficial stimuli (e.g., pH/ROS, pH/redox, enzyme/ROS, or pH/redox/temperature), making the system more adaptable to variations in the disease environment. Advanced engineered mechanisms: cascade activation, where the first pathological stimuli induce a chemical-physical transformation that triggers the exposure of a second response stimulus, is one important mechanism; AND- or OR-logic gate operations applied to nanocarriers allow enhanced specificity (AND gate when both triggers must be present to release drug) or increased robustness against different disease states (OR gate). Additionally, functional transformations may improve biological activity. For example, Charge-reversalnanoparticlesare neutral or negative in blood but positive in diseased tissues, leading to enhanced cellular interaction and internalization. Size-switchable nanoparticles may dynamically adjust their sizes to simultaneously satisfy long circulation time and deep tissue penetration. In addition, shape transformation of nanocarriers may facilitate their retention and cellular uptake at the disease sites and can also control their circulation. Lastly, targeting toward desired receptors in disease by polymer coating, aptamers, antibodies, peptides, small molecule ligands, carbohydrates, or even other specific proteins could also be enabled by surface functionalization with a targeted moiety (Figure 3). As such, by programming the choice of stimulus in a precise manner, controlled stimulus responses in serial or parallel, the logic-based functionality and stimuli-driven transformations in charge, shape, and size, as well as the targeting specificity to diseases, will enable advanced smart nanocarriers for precision therapy[10].

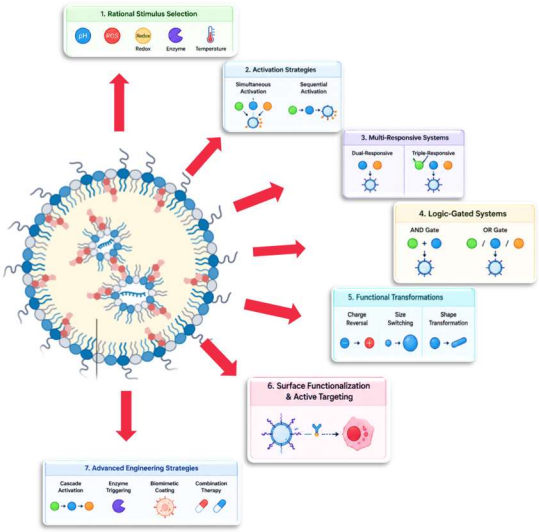

Fig.3. Design principle and engineering strategy of multi-stimuli-responsive nanoparticles

Materials, Nanocarrier Platforms and Stimuli-Sensitive Functional Components

The functionality of multi-stimuli-responsive nanoparticles is highly influenced by the choice of carrier material, nanoscale structure, and stimulus-sensitive functional units. The use of natural polymers, including chitosan, alginate, dextran, hyaluronic acid, gelatin, and cellulose derivatives, provides for good biocompatibility and degradability and is associated with rich functionalities that allow chemical modification. The controlled delivery of diverse small-molecule drugs, proteins, and nucleic acid drugs may then become a reality by assembling such materials into various polymeric self-assembled nanoparticles, micelles, nanogels, and dendrimers composed of polymer materials, such as PLGA, PCL, PEG, PNIPAM, and poly(amino esters)s with fine control over molecular weights, degradation rates, surface properties, and triggered stimuli. The lipid-based systems like liposomes and lipid–polymer hybrid nanoparticles benefit from biocompatibility and high loading capacity of varied therapeutics while being stimuli responsive to variations of pH, redox, enzymes or temperature[11].

Polymeric micelles are especially effective in encapsulating hydrophobic molecules whereas nanogels with higher water content may be tailored for variable swelling and deformity. Dendrimers with finely structured branches and dense surface group populations can be prepared. The inorganic nanomaterials, which include mesoporous silica nanoparticles, metal nanoparticles, and the magnetic nanoparticles, may possess high specific surface area, pore size and volume, adjustable imaging properties, photothermal properties, responses to an external magnetic field, or a high pore size with programmable complex chemistry and coordination chemistry for the metal-organic frameworks. Biomimetic nanomaterials like ones coated with cell membranes, extracellular vesicle contents, and natural derivatives can improve the immunity-evading property, prolong circulation, and to recognize target tissues specifically[12].

Stimuli-sensitive functionalities are commonly achieved by employing smart cleavage linker groups, such as hydrazone, acetal, disulfide, thioketal, boronate ester, peptide, and hypoxia-sensitive bonds. The integration of a range of these smart materials and devices opens avenues for programmable, disease-specific, and even sequential delivery of therapies.

Optimization

Multi-stimuli-responsive Nanoparticle Fabrication. The design of multi-stimuli-responsive nanoparticles requires facile synthesis methodologies to enable fine-tuning of particle size, shape, drug loading, surface functionalization, colloidal stability, and responses. By exploiting the electrovalent bonding of oppositely charged polymers to attain soft polymer formation by thin ion gelation. of nanoparticles from polymers, this is a preferred method because solvent diffuses out quickly, thereby allowing easy and quick formation of nanoparticles under gentle conditions with minimal structure changes. The encapsulation of both hydrophilic and hydrophobic drugs into the microparticles can be achieved through both single and double emulsion systems[13].

Self-assembly that relies on amphiphilic properties leads to the formation of micelles, vesicles, and other nanostructures.

The still important role in the preparation liposome is the thin film hydration method. Thin ion gelation can achieve the soft preparation of polymer through electrovalent interactions between differently charged polymers. Some novel fabrication approaches can offer superior controllability and Reproducibility of creating nanostructures. Microfluidic processes facilitate quick mixing and narrow particle size distribution; moreover, they can implement continuous processing and regulation of multiple process variables.

Layer-by-layer assembling (LbL) permits the stepwise stacking of polymers or Functional materials, resulting in Multi-Component architecture.

Conjugation with covalent or Affinity bonds to form surface modification on Nanoparticle couldimmobilizethe targeting ligands (antibody, peptide, aptamer, targeting polymer), stimulus-sensitive polymers and protein and etc. Scaling up of nanoprecipitation, microemulsion, self-assembly, and ionic gelation methods to yield Nanoparticles with excellent Batch-to-batch consistency, including high-pressure homogenization, continuous-flow processes,

controlled mixing methods, and membrane technology[14].

Quality by Design framework is important for optimization as it involves interacting multiple formulation and process variables. The whole process includes: establishing the Quality Target Product Profile, identifying the Critical Quality Attributes (CQAs), Critical Material Attributes (CMAs), and Critical Process Parameters (CPPs), and performing risk assessment for selecting influential variables with tools such as Failure Mode and Effects Analysis (FMEA) or Fishbone Diagrams (Ishikawa diagrams). Experimental Designs such as Factorial Design (full factorial or fractional factorial), Box-Behnken design, or Central Composite Design should be used to investigate and optimize, and eventually establish a multidimensional design space for robust manufacturing.

Physicochemical Characterization and Evaluation of Multi-Stimuli Responsiveness

Particle Size: Nanoparticle size: Multi-stimuli responsive nanoparticles are very important due to loading of drug, behaviour of circulation, uptake by cell, uptake in tissue and distribution. It can be monitored by DLS, supplemented by electron microscopic observations, to check the size and degree of aggregation of primary particles[15].

Polydispersity Index (PDI): The polydispersity index (PDI) represents the width and homogeneity of particle size distribution of the sample. Typically, PDI=1 means that the sample consists of only one single particle size. High values may point to large size dispersion and aggregate formation, leading to instability, low reproducibility, and poor stimuli-responsiveness[16].

Zeta Potential: A measure of nanoparticle surface charge and electrostatic stability, zeta potential can also provide valuable information. Zeta Potential, Size, Size & Charge Distribution: The absolute values of zeta potential can range from -1 to -30mV and -1 to +30 mV is considered the acceptable range, though many systems fall within -50 to +50mV. Small absolute values usually mean that the system will agglomerate; however, changes to zeta potential in the presence of enzyme, pH shift, changes in redox potential, and other conditions can be useful indices of surface cleavage and restructuring[17].

Morphology: Typical techniques to characterize the particle morphology include Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and atomic force microscopy (AFM). All of these can be applied to good analysis of the size, shape, and the textural characteristics of surfaces, and even arrangement and structure within the particle. The methods are used as well in the study of changes in form, or of response to swelling, breaking up, and swelling and breakage[17].

Drug Loading: Drug loading describes how much active ingredient is loaded as a function of the mass of the total particle formulation. Ideally, drug loading is high to avoid carrier loading and maximize therapy, but when exposed to a stimulus, non-release conditions must be validated to avoid premature loss of drug[18].

Encapsulation Efficiency: The encapsulation efficiency (EE) is the percentage of drug trapped into the nanoparticles among the loaded amount, which varies as a function of the drug physicochemical properties, the characteristics of the carrier, and the fabrication procedure. EE can be determined by HPLC, UV–visible spectrophotometry, or another justified analytical technique[19].

Surface Chemistry: The presence of functional group coatings, targets, and sensitive linkers is confirmed by the characterization of the surface chemical features (FTIR, XPS, NMR spectroscopy can be used to monitor conjugation, surface modifications, and stimulus-responsive chemistry)[19].

Thermal and Solid-State Characterization: Physical characterization (DSC, TGA, XRD was used to determine the thermal properties, crystallinity, phase transitions, and drug-excipient interaction. Drug status in carrier (crystalline, amorphous, or molecularly dispersed). The physical characteristics determine how the drug exists within the carrier[20].

Colloidal Stability: The colloidal stability is assessed by measuring particle size, PDI, zeta potential, aggregation, precipitation, and drug leakage in storage as well as in physiological media. Stability in serum-containing media is the one most representative for *in vivo* conditions.

Stimulus Threshold: The threshold stimulus refers to the minimum levels/concentration of trigger that causes a detectable response from the nanoparticle. The ability to set thresholds of parameters such as pH, reactive oxygen species, glutathione, enzymes, temperature, light, magnetic fields, and ultrasound is crucial to differentiating normal physiological and pathological activation.

Response Kinetics: The rate of physicochemical transformations within nanoparticles in response to one or more stimuli. Parameters included response lag time, activation rate, swelling rate, bond-cleavage rate, disassembly time, and drug-release rate.

Reversibility: Reversibility assesses the degree to which changes in the stimuli return to their original state after removal of the stimulus. Reversible systems can repeatedly cycle between states such as swelling–deswelling, association–dissociation, or charge-switches, whereas irreversible systems mainly require a permanent cleavage of bonds or depletion of carriers.

Structural Transformation: Structural transformation studies monitor changes in the architecture of the nanoparticles post-stimulation, such as swelling, shrinking, pore formation, shelling, change in charge, disintegration, or fragmentation. Structural transformation of nanoparticles can be monitored by DLS, TEM, cryo-TEM, spectroscopy, scattering, and release studies.

Sequential or Simultaneous Trigger–Response Studies: Testing should include individual, serial, and combinatorial triggering of multi-stimuli-responsive nanoparticles. Sequential studies identify the potential for the introduction of a particular stimulus to potentiate or trigger a response to a second stimulus, while serial studies investigate synergy, additivity, or antagonism of the interaction between different pathogenic or exogenous stimuli.

***In Vitro*, *Ex Vivo* and *In Vivo* Performance Evaluation**

A complete, multi-scale, integrated assessment from the *in vitro* to the *ex vivo* and *in vivo* level is, therefore, fundamental to define a real structure-function-efficacy-safety profile of a multi-stimuli-responsive nanocarrier and move toward clinical applications. Therapeutic effects have to be achieved in disease-relevant animal model(s), especially those chronic disease models, compared to the counterpart free drugs, non-responsive carriers, and single stimuli-responsive carriers. Many applications, including in vascular targeting, require some level of both biocompatibility and haemocompatibility; a variety of standard blood-compatible tests, such as hemolysis, coagulation time, and complement and platelet activation assays, exist for evaluating both. Various pH, various redox potential, varying ROS concentrations, varying enzymatic concentrations, various oxygen concentrations (hypoxia levels), various glucose concentrations, varying temperatures, lights, magnetic or acoustic energy. The kinetics of release should finally be analysed using appropriate models corresponding to different release mechanisms (e.g., diffusion, erosion, swelling, degradation, or a combination of mechanisms) and checked if this release happens sequentially, simultaneously, or in a logic-gated manner. Cellular level assessment includes nanocarrier cellular uptake efficiency, receptor-mediated internalization, endosomal escape, intracellular trafficking and targeting (e.g., in specific organelles), and stimulus-induced intracellular drug delivery in the cytoplasm or specific organelles. Various flow cytometric and microscopy-based assays should be employed for this purpose. Cell biocompatibility and biological activity can be evaluated based on *in vitro* toxicity (e.g., CC50 value), cell viability, apoptosis, oxidative stress, cell membrane integrity, and biological function-based assays. Biocompatibility and haemocompatibility are crucial in many applications (e.g., vascular targeting) and can be assessed using a series of standard blood-compatible tests, such as hemolysis, coagulation time, platelet activation, and complement activation assays. *Ex vivo* tissue models could provide a valuable intermediate tool to mimic the *in vivo* environment to study drug penetration into tissues, vascular permeation and retention, barrier transport, and drug distribution within a 3D structure.

In vivo evaluation involves detailed assessment of the pharmacokinetics (PK), including drug half-life, total exposure, clearance, and bioavailability, and Quantitative biodistribution and targeted organ accumulation of the nanoparticles. Pharmacodynamic (PD) studies correlate the PK parameters of nanocarriers with disease-relevant biological responses, therapeutic outcomes, and specific biomarkers. Therapeutic effects have to be demonstrated in relevant disease animal models, usually chronic disease models, and compared with the equivalent free drugs, non-responsive carriers, and single-stimulus-responsive carriers. Final safety assessment, including acute and repeated dose toxicity studies, histopathology analysis of multiple organs, hematology, clinical chemistry, immunogenicity studies, and chronic toxicity testing, is all crucial and mandatory. Therefore, a comprehensive integrated evaluation from *in vitro* through *ex vivo* to *in vivo* stages is necessary to establish a meaningful structure-function-efficacy-safety relationship of a multi-stimuli-responsive nanocarrier and thus enable clinical translation.

Disease-Specific Applications in Chronic Disorders

A Variety of nanomaterials with multiple stimuli-responsive properties have extensive therapeutic potential for treating chronic diseases that feature highly complex pathologies with dynamically changing heterogeneity in micro-environments. Smart

nanoplatforms capable of sensing tumor environments with acidic pH, ROS, glutathione, hypoxia, and enzymes facilitate the delivery of tumor-selective drugs into the target area, enhance penetration into solid tumors, improve intracellular uptake and accumulation, enable combination chemotherapy, and reduce off-target toxicity. In diabetes, the system may be responsive to triggers and also be responsive in some other aspect, pH, ROS, or enzymes, which provides you with smart delivery of a glucose-responsive system. It can be used in combination with oxidative stress management or inflammation management, or it could also be combined with management of impairment of the tissue regeneration process. Cardiovascular disease involves the use of pH/ROS, enzyme/ROS, and shear-responsive nanoparticles to target atherosclerotic plaques, ischemic heart tissues, blood clots, and vascular inflammation. In rheumatoid arthritis and other chronic inflammation disease, the combination of triggers like ROS, pH, protease, and inflammatory cytokines provides a good platform for site-selective delivery of anti-inflammatory drugs, biologic agents, and nucleic acids in an attempt to deliver those agents into diseased areas selectively. To cure neurodegenerative disease, responsive nanoparticles should not only address the challenge of getting through the blood-brain barrier but also the ROS, abnormal enzymes, protein aggregation, and redox changes in intracellular conditions. In CKD patients, the appropriate utilization of nanoparticles responsive to pH, ROS, enzymes, fibrotic microenvironments, and shear can reduce the systemic delivery of the therapeutic agents and enhance their concentration at the target tissue in the kidney while addressing chronic renal inflammation, oxidative injury, fibrosis, and impaired enzyme activities. Liver disease can be well addressed with nanoparticles responsive to ROS, pH, enzymes, and the fibrotic microenvironment that target hepatocytes, macrophages, and activated hepatic stellate cells[21]. Chronic lung disease patients can be treated with inhaled nanoparticles that are responsive to the mucus, ROS, enzymes, and inflammatory pH to improve retention in the lung and local treatment (Table 1). In chronic wound therapy, nanocarriers with glucose-, ROS-, pH-, enzyme-, temperature- or infection-responsive capabilities are designed to enable sustained delivery of antimicrobial agents, modulate local inflammation, and promote tissue repair and regeneration by promoting new blood vessel formation and the tissue repair process.

Table 1: Applications and Evaluation of Multi-Stimuli-Responsive Nanoparticles in Chronic Diseases

Chronic disease	Pathological stimuli exploited	Nanoparticle system	Therapeutic objective	Key evaluation parameters	Major translational challenge	Reference
Alzheimer's	ROS, enzymes, pathological proteins	BBB-target	Brain targeting	BBB penetration, cognition, and bioavailability	BBB complexity	[22]
Atherosclerosis	ROS, enzymes, acidic microenvironment	Inflammation-responsive	Plaque-specific delivery	Plaque accumulation and inflammation	Target specificity	[23]
Diabetes mellitus	Glucose, pH, ROS	Glucose-responsive	Feedback-regulated insulin discharge	Glucose responsiveness	Mutable glucose dynamics	[24]
Inflammatory bowel disease	pH, ROS, enzymes	Colon-targeted	intestinal targeting	Mucosal accumulation	Adjustable GI environment	[25]
Kidney disease	ROS, enzymes, pH alterations	Renal-targeted responsive	Kidney targeting	Renal accumulation	Rapid clearance	[26]
Liver	ROS, enzy	Hepatic	Antifibr	Liver	Hepatic	[27]

disease	mes, inflammatory signals	responsive	otic delivery	accumulation, fibrosis markers	sequestration	
Lung disease	ROS, enzymes, pH	Inhalable responsive	pulmonary therapy	Lung deposition	Mucociliary clearance	[28]
Malignancy	Acidic pH, ROS, GSH, hypoxia, enzymes	Dual/triple-responsive	Tumor targeting	Tumor accumulation, apoptosis, and drug distribution	Tumor heterogeneity	[29]
Parkinson's	ROS, oxidative stress, enzymes	Redox-responsive	Neuronal targeting	Brain accumulation	Neurotoxicity	[30]
Rheumatoid arthritis	ROS, acidic pH, enzymes	ROS/pH-responsive	Joint targeting	Cytokines	Chronic immune activation	[31]

Translational, Manufacturing and Regulatory Challenges

The development and validation of methods for complex multiple stages and stimuli-responsive behaviour, alongside a detailed roadmap on the proper design of these complex nanomedicines to achieve reliable and reproducible therapeutic profiles and safety, remains essential for successful clinical translation in the future. Premature release of drugs in the circulation further limits efficacy and can enhance the risk of systemic toxicity. Scalability issues on mixing, mass transfer, self-assembly and morphology, and the effects of sterile technologies on nanoparticle stability and reactivity[32]. Recognition by the mononuclear phagocyte system, followed by rapid clearance through the immune system, could further reduce circulation times and lead to non-specific accumulation in the spleen or liver. A key drawback of the proposed design lies in combining the stimuli-responsive polymers, biodegradable linkers, targeting ligands, and hierarchical structures together. Batch-to-batch consistency is difficult in PDI, sizes, drug loading, surface functional groups and size distributions, response kinetics, and stimulus responses. Challenges of scaling up include mass transfer, mixing, self-assembly morphology, influences of sterilization on morphology stability and responsiveness. Premature release of encapsulates could happen in blood circulation systems. The potential toxicological issues of non-biodegradable residues and their long-term accumulation, the immunogenicity and complement activation by such nanoparticles, as well as toxicity from their degradation products need to be addressed. The development and validation of methodologies to assess complex, multi-stage and multi-stimuli-responsive behaviors, coupled with clear guidelines for the rigorous development of such advanced nanomedicines to establish reliable and reproducible therapeutic performance and safety profiles, is imperative for future clinical translation.

Emerging Trends and Future Perspectives

The design of multi-stimuli-responsive nanoparticles is being pushed towards smart, predictable, clinically applicable drug delivery[33-40]. The integration of AI to help with nanoparticle design and ML-based optimization will reduce formulation time by elucidating complicated interrelationships between material properties, process conditions, CQA's, biological responses, and therapeutic effects. The development of data-driven models could potentially guide rational selection of the trigger, predict nanoparticle behaviour, optimize multi-component formulation, and minimize empirical testing. The use of enhanced pre-clinical tools such as patient-derived organoids and OOC will better mimic physiological conditions and could improve our assessment of NP penetration into target tissue, overcome biological barriers, analyze toxicity, and evaluate stimuli-triggered NP function compared to traditional 2D cell-based studies.

A second trend in multi-stimuli responsive NPs is towards theranostic nanoplatforms capable of image-guided drug delivery, disease monitoring, and targeted therapeutic release. The next steps involve personalization of NP's where NPs might be engineered based on the patient and their condition, with optimized carrier composition, targeting ligands, stimulus thresholds, and dosing regimens. Another upcoming development is a self-regulated closed-loop delivery system for the treatment of chronic diseases, which will sense a certain biomarker of disease

activity and respond with automatic delivery. Furthermore, programmable logic gate NPs based on OR, AND, cascading, or sequential operation mechanisms can enhance target specificity and can integrate and process multi-biomarker inputs before delivering their payload. Development of digital twins and systems biology modeling will help predict NP behavior, patient outcomes, and treatment results in a virtual world. However, the future of these multi-responsive systems is also determined by the development of clinically friendly multi-stimuli systems that don't complicate manufacturing and verification and will make approval easier compared to the extremely complicated systems developed today. Researchers will have to focus on validation of the multi-trigger responses with well-established and standardized testing methods, develop predictive structure-function models, and use data to demonstrate a favorable risk-to-benefit ratio and therapeutic superiority in clinical settings, as well as scale up for industrial application, safety assessments, and establish clinically relevant disease models and markers for disease response validation. The recent patents and marketed products on multi-stimuli-responsive nanoparticles are shown in Tables 2 and 3.

Table 2: Representative patents related to multi-stimuli-responsive nanoparticles for targeted drug delivery

Patent No. and year	Grantee	Novelty	Target	Applications
EP4630061A1; 2025	Nanovation Therapeutics Inc.	Lipid nanoparticles	Ligand-mediated targeting to the liver	Chronic inflammatory-based diseases, liver cirrhosis, genetic diseases.
AU2023387789A1; 2024		lipid target-specific nanoparticles	Tissue-specific targeting	Autoimmune diseases, chronic metabolic conditions.
WO2024086929A1; 2024		Lipid nanoparticles	pH-responsive release	Diseases that affect the brain and the nerves
US20240285544A1; 2024		lipid nanoparticles	pH-triggered release	Chronic liver/ heart disorders
AU2023406303A1; (WO2024119039); 2024	Moderna Inc.	Lipid nanoparticles	Immune evasion	Chronic inflammatory diseases, gene therapy
US20100209492010	SDG Inc.	Liposomes	Ligand-mediated	Cancer and inflammatory diseases
US20070104777 2007	Lau et al.	Liposomes	Receptor-mediated	cancer and chronic diseases
US20070286898 2007	Astellas Pharma	Liposomes	stimulus-responsive delivery	Chronic and inflammatory conditions

Table 3: Representative marketed nanoparticle-based products relevant to targeted and stimuli-responsive drug delivery

Name	Main content	innovation	manufacturer	year	Indication	Significance
Doxil	Doxorubicin	PEGylated liposome	Janssen	1995	Ovarian cancer, Kaposi's sarcoma	tumor targeting
Onpattro	Patisiran	Lipid nanoparticle	Alnylam	2018	Hereditary transthyretin amyloidosis	nanoparticles
Abraxane	Paclitaxel	Albumin nan	Bristol Myers	2005	Breast/pancreas/pulmonary	nanoparticles

		opar ticle s	Squi bb		carcinom as	
Ari kay ce	Ami kaci n	Lip oso mal inha latio n	Insm ed	2 0 1 8	Pulmona ry diseases	Alv eoli targ etin g
Co mir nat y	mR NA	Lipi d nan opar ticle s	Pfize r	2 0 2 1	COVID-19	intra cell ular deli very
Spi kev ax	mR NA	Lipi d nan opar ticle s	Mod erna	2 0 2 2	COVID-19	chro nic dise ases
Am Bis om e	Am phot erici n B	Lip oso me	Gile ad Scie nces	1 9 9 7	fungal ailment	Alv eoli targ etin g
Ab elce t	Am phot erici n B	Lipi d com plex	Sig ma-Tau	1 9 9 5	fungal diseases	Lipi d-base d targ etin g

Conclusion

One important improvement in this area involves multi-stimuli-responsive nanoparticles that promise adaptive, tightly regulated responses to dynamically changing, complicated microenvironments present in chronic diseases. Instead of using conventional drug delivery formulations and single-stimulus-responsive nanoparticles, such platforms use various types of native or introduced stimuli to achieve targeted accumulation at the site of the disease, enhance cell uptake, facilitate cellular transport, and control the timing of drug release. Using such stimulus combinations in a carefully reasoned manner, e.g., pH/redox, pH/ROS, enzyme/ROS, pH/temperature, pH/light, etc., may lead to better spatio-temporal targeting while preventing early drug release and unintended side effects on normal tissues and organs. New types of nanoplatforms are even emerging, such as those based on a cascade response, sequential activation, logic gates, charge switching, size change, shape change, etc., allowing for extremely specific and programmably tailored therapies. The importance of multi-stimuli-responsive nanoplatforms for the management of chronic diseases that are associated with often highly heterogeneous and continuously changing pathological conditions like cancer, diabetes, chronic cardiovascular diseases, inflammatory disorders, chronic neurodegenerative diseases, chronic kidney, liver, and lung diseases, etc. Is thus very apparent. It must, however, be stressed that for effective design, the selection of appropriate stimuli combination should match well with disease-specific biology rather than solely be driven by the complexity of the nanoparticle. Similarly, the choice of materials should also consider other important factors such as biocompatibility, degradability, stability, compatibility with the drug, manufacturing feasibility, and safety with a long-term perspective. Although the therapeutic effectiveness has shown promise in many preclinical settings, translational challenges such as heterogeneity of the diseases, variation in stimulus intensity, premature triggering, protein corona effect, immune response, lack of biodegradation, long-term toxicity, challenges in scale-up, batch-to-batch variations, and regulatory complexities remain. Future efforts may therefore be directed to develop simpler yet functional nano-systems, standardize methodology for quantifying the stimulus intensity and response rates, and validate performance under single as well as individual/mixed, consecutive, and simultaneous triggering. Use of more relevant and predictive experimental models such as 3-D cell cultures, organoids, microfluidic platforms, and organs-on-a-chip is needed to improve translation potential. Integration with AI/ML algorithms, microfluidics-based manufacturing, theranostics approach, live imaging techniques, and personal medical information for personalized therapy may be considered as other directions for development.

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