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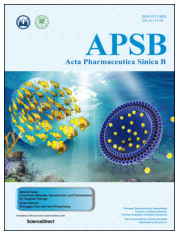

REVIEW

Charge-reversal nanoparticles: novel targeted drug delivery carriers

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Abstract Spurred by significant progress in materials chemistry and drug delivery, charge-reversal nanocarriers are being developed to deliver anticancer formulations in spatial-, temporal- and dosage-controlled approaches. Charge-reversal nanoparticles can release their drug payload in response to specific stimuli that alter the charge on their surface. They can elude clearance from the circulation and be activated by protonation, enzymatic cleavage, or a molecular conformational change. In this review, we discuss the physiological basis for, and recent advances in the design of charge-reversal nanoparticles that are able to control drug biodistribution in response to specific stimuli, endogenous factors (changes in pH, redox gradients, or enzyme concentration) or exogenous factors (light or thermos-stimulation).

Abbreviations: Abs, integrin aVb3 mAbs; B-PDEAEA, poly[(2-acryloyl) ethyl (*p*-boronic acid benzyl) diethylammonium bromide]; BPS, bridged polysilsesquioxanexerogel; BSA, bovine serum albumin; CA4, combretastatin A4; CAPL, charge reversible pullulan-based; CHPNH2, cationic cholesteryl group–bearing pullulans; Cit, citraconic anhydride; CMC, carboxymethyl cellulose; CPLAs, cationic polylactides; Cya, cysteamine hydrochloride; DAP, 2,3-diamino-propionate; DCL, dimethyl maleamidic acid-*ε*-caprolactone; DDS, drug delivery system; DM, dimyristeroyl; DMA, 2,3-dimethylmaleic anhydride; DMPA, dimethylol propionic acid; DOX, doxorubicin; FITC, fluorescein isothiocyanate; Glu, glutamic acid; GO, graphene oxide; GSH, glutathione; HCC, hepatocellular carcinoma; HEP, 1,4-bis(2-hydroxyethyl) piperazine; His, histidine; HMP, *p*-hydroxylmethylenephenol; MG, microgels; MMPs, matrix metalloproteinases; MNP, magnetic nanoparticles; NPs, nanoparticles; PAEP, poly(allyl ethylene phosphate); pA-F, fluorescein-labeled polyanion; PAH, poly(allylamine) hydrochloride; PBAE, poly(*β*-amino ester); PCL, poly(*ε*-caprolactone); PDADMAC, poly(diallyldimethylammonium chloride); PEG, polyethylene glycol; PEI, polyethylenimine; PEO, poly(ethylene oxide); PK, protein kinase; PLA, ploylactic acid; PLGA, poly(lactic-*co*-glycolic acid); PLL, poly(L-lysine); PMA, poly(methacrylic acid); PS, pH sensitive; PSS, poly(sodium 4-styrenesulfonate); PSSS, poly(styrene-*co*-4-styrene-sulfonate); PTX, paclitaxel; PU, polyurethane; PVPON, poly(*N*-vinylpyrrolidone); ROS, reactive oxygen species; SOD, superoxide dismutase; TMA, 2-(mercaptoethyl) trimethylammonium chloride; TUNA, thioundecyl-tetraethyleneglycolester-*o*-nitrobenzy-lethyldimethyl ammonium bromide

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1. Introduction

Cancer is a leading cause of death around the world. According to the mortality data from World Health Organization in 2015, there were an estimated 84 million cancer deaths in the last decade[1]. Cancer development has been defined as a multistep process by which an initiating event (*e.g.*, environmental insult) leads to malignant proliferation. As a small tumor mass forms, the surrounding healthy tissue is unable to compete with the cancer cells for an adequate supply of nutrients from the blood system, leading to apoptosis and necrosis of the normal cells followed by dysfunction of primary organs and death[2]. Current therapeutic strategies for most cancers involve a combination of surgical resection, radiation therapy, and chemotherapy. However, significant morbidity and mortality are always associated with these therapies due to their off-target effects on the "normal" cells. The efficacy of a chemotherapy regimen is directly correlated with the ability to selectively target tumor tissue, overcome biological barriers, and "smartly respond" to the tumor environment to release therapeutic agents[3]. In conventional drug delivery, the drug is exposed directly to serum without protection. The drug concentration in the blood increases rapidly after administration and then declines. The purpose of an ideal drug delivery system (DDS) is to adjust the drug concentration within a desired therapeutic range after a single dose, and carry the drug to a targeted region while simultaneously lowering the systemic levels of the drug[4]. Charge-reversal nanoparticles exert significant potential for the specific targeting and release of anti-cancer drugs. Nanoparticles are defined as submicronic colloidal systems. Nanosized drug carriers have a variety of intrinsic advantages over conventional drug delivery systems, such as large payload capacity for anticancer formulations, protection from degradation, multivalent targeting moieties, and controlled or sustained release that reduces adverse effects while enforcing the safety margin of the antitumor agents[5–7]. Nanoparticles are usually taken up by various metabolic systems depending on their surface characteristics. Generally, the positive charge facilitates the binding of nanoparticles to the cell membrane, leading to a significant improvement in membrane transport properties because of the intrinsic negative surface charge of the cell membrane. However, this positive charge might also strengthen the nonspecific binding of vectors to normal tissue[8]. The luminal surface of blood vessels is well known to have a negatively charged surface contributed by sulfated and carboxylate sugar moieties, meaning that nanoparticles with high positive charges will bind nonspecifically to the luminal surface of vascular walls and be rapidly cleared from the blood circulation[9]. Charge-reversal nanoparticles combine the targeting advantages of a conventional "smart" nanoparticle with a charge-switch characteristic for drug release. Surface charge is designed to be obscured during the blood circulation and uncovered at tumor sites. Thus, these novel anti-cancer drug carriers have attracted tremendous attention for delivery of anticancer agents. Herein, we provide a brief review of several possible targeting delivery strategies for charge-reversal nanoparticles.

2. Endogenous stimuli-responsive charge-reversal delivery

2.1. *pH-triggered charge-reversal delivery*

Low cellular pH has been widely used to design sensitive drug delivery strategies. Previous reports demonstrated that pH values vary significantly in different tissues or organs, (such as stomach and brain), and in morbid states, (such as diabetes, infection, inflammation, and tumor)[10]. The pH in tumor tissue is lower than that in normal tissues because of the high rate of glycolysis in cancer cells. Compared with the pH 7.4 of normal tissue, the pH in a tumor has been demonstrated to range from 5.7 to 7.8. Additional pH differences are observed at the subcellular level. The late endosomes and lysosomes have a much lower pH, in the range of 4.5–5.5. Several drug carriers are absorbed through endocytosis and assimilated within endosomes and lysosomes. This pH gradient is significant for cancer drug delivery. pH-Sensitive nano-systems are designed to stabilize the cargo at physiological pH, and release the drug rapidly when the pH triggering-point is reached (Fig. 1A). Kim et al.[11] synthesized a

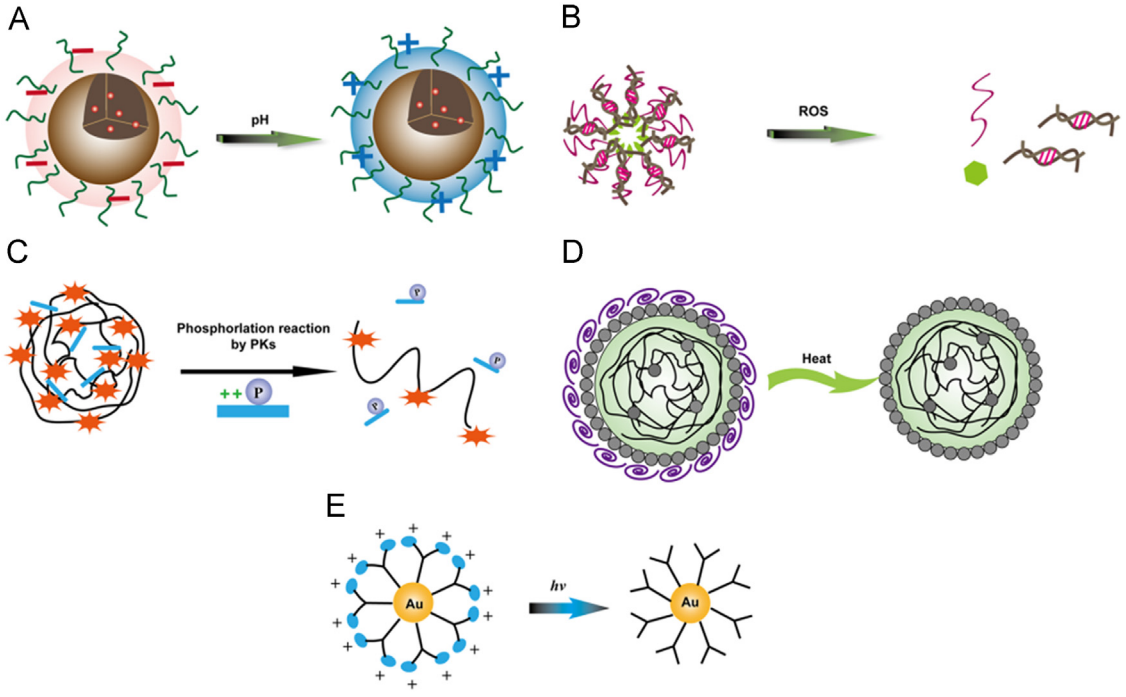

Figure 1 Endogenous and exogenous stimuli-responsive charge-reversal delivery. (A) pH-triggered charge-reversal delivery; (B) tumor redox environment–triggered charge-reversal delivery; (C) tumor protease-triggered charge-reversal nanoparticles; (D) light-triggered charge-reversal drug delivery; and (E) thermo-responsive charge-reversal drug delivery.

poly(ethylene oxide)-*b*-poly(methacrylic acid) (PEO-*b*-PMA) copolymer with doxorubicin (DOX) incorporated into the ionic cores of PEO-*b*-PMA micelles *via* electrostatic interactions. DOX is positively charged at physiological conditions. The ammonium group in the daunosamine part of DOX and the hydrophobic interactions between the anthracycline residues of DOX provide stabilization of the complex. The protonation of a carboxylic group in the core of the micelles leads to DOX release at lower pH. Up to 50% of the DOX was released during the first hour at pH 5.5. Yu and coworkers[12] synthesized mPEG-PU(HEP-*co*-DMPA)-mPEG polyurethane triblock copolymers (PS-PUs). The zeta potential showed a charge-reversal point at about pH 5.7, 6.4, and 6.9 for PS-PU2, PS-PU3 and PS-PU4. It suggested that the transition pH of PS-PUs was tunable by changing the molar ratio of piperazine/carboxyl, where the tertiary amino groups were protonated and generate an isoelectric point with carboxylic acid groups at a specific pH. Wang and colleges[13] demonstrated a stepwise pH-responsive nanoparticle system containing charge reversible pullulan-based (*i.e.*, CAPL) shell and poly(β-amino ester) (PBAE)/poly(lactic-*co*-glycolic acid) (PLGA) core. This nanoparticle system was designed as a carrier of paclitaxel (PTX) and combretastatin A4 (CA4) for combining antiangiogenesis and chemotherapy to treat hepatocellular carcinoma (HCC). CAPL-coated PBAE/PLGA (CAPL/PBAE/PLGA) nanoparticles displayed a step-by-step response to weakly acidic tumor microenvironment and endo/lysosome through the cleavage of β-carboxylic amide bond in CAPL and the "proton-sponge" effect of PBAE, thus achieving the efficient and orderly releases of CA4 and PTX.

2.2. *Tumor redox environment-triggered charge-reversal delivery*

During the process of intracellular oxygen metabolism, immune-system attack on pathogens, as well as a number of human pathological conditions, free radicals and reactive oxygen species (ROS) present and harm the human internal environment. Multiple mechanisms or processes exist to protect against free radicals and ROS. However, these protective mechanisms may be overwhelmed or inefficient in handling free radicals/ROS, leading to "oxidative stress". The main protective mechanisms against ROS include superoxide dismutase (SOD), catalase, glutathione (GSH), protein thiols, and other intracellular redox couples[14]. These processes constitute a complex intracellular network designed to maintain a slightly reducing environment. Many researchers have utilized this cellular characteristic for the targeted and charge-reversal delivery of anti-tumor drugs (Fig. 1B). Caruso et al.[15] employed a layer-by-layer technique to construct a charge-reversal nanoparticle which can facilitate the absorption of another layer of oppositely charged polyelectrolyte. They constructed poly(*N*-vinylpyrrolidone) (PVPON) and poly (methacrylic acid) (PMA) capsules. The disulfide linkages were much more stable in a normal physiological environment, such as the bloodstream, and were cleaved under reducing conditions such as the cytoplasm of tumor cells. Shen and coworkers[16] constructed a reactive oxygen species labile charge-reversal polymer, poly[(2-acryloyl) ethyl (*p*-boronic acid benzyl) diethylammonium bromide] (B-PDEAEA). The polymer was strongly positively charged to which DNA could be effectively trapped and compressed into nanoparticles. The polymer tends to become negatively charged after triggering by intracellular ROS. The quaternary ammonium compound released *p*-quinone methide (*p*-hydroxylmethylenephenol, HMP) and leaving behind a tertiary amine following oxidation of the boronic acid group by ROS. It subsequently auto-catalyzed fast hydrolysis of the ester group producing poly(acrylic acid).

2.3. *Tumor protease triggered charge-reversal nanoparticles*

Mounting evidence supports the point that extracellular proteases, such as the matrix metalloproteinases (MMPs), regulate many biochemical factor changes in the microenvironment during tumor progression[17]. These proteases mediate various physiological processes and signaling pathways and thus they play a key role in signal communication between tumor cells and the extracellular environment (Fig. 1C).

Andresen et al.[18] constructed a novel lipopeptide–poly(ethylene glycol) (PEG) conjugate composed of a lipid-anchor, a peptide sensitive to MMP2, and a PEG chain. The amphiphilic molecule consists of dimyristeroyl (DM) conjugated to 2,3-diamino-propionate (DAP), which is conjugated to the N-terminal of peptide WIPVSLRSGEEEE, and then to PEG 2000 through its C-terminal. After cleavage of the peptide by MMP2, the charge reverses from negative to positive at lower pH. Katayama and co-workers[19] constructed protein kinase (PK)-responsive nanoparticles (NPs) comprising a hydrophobically modified peptide substrate for PKs and a fluorescein-labeled polyanion (pA-F). Initially, the fluorescence of fluorescein was largely quenched due to self-quenching within lipopeptides and pA-F aggregates. However, PK-catalyzed phosphorylation of cationic lipopeptides can reverse nanoparticle charge and dissociate nanoparticles, as evidenced by the prominent fluorescence emission recovery.

3. Exogenous stimuli-responsive charge-reversal delivery

3.1. *Light-triggered charge-reversal drug delivery*

Various physical and chemical stimuli as well as their combination can switch the modified electrode interface of nanoparticles. There are active and inactive states for electrochemical, electrocatalytic and bio-electrocatalytic reactions. The electron transfer from these reactions may be applied for charge-reversal drug delivery systems. Light, a noninvasive approach, is a quite attractive trigger for delivering anti-cancer agents capable of rapid and precise release. Since the 1980s, photodynamic therapy has served as a promising treatment method which involves the utilization of a photosensitizing agent coupled with an appropriate light source. Although only a few methods have made it to the clinic, many photo-sensitizing agents have been found in the laboratory[20]. Light-responsive charge-reversal nanoparticles are used as a microcarrier to deliver different drugs into cells[21]. A wide range of research is currently under study to optimize the light-responsive materials to achieve therapeutically efficient and reproducible release profiles.

Shea et al.[22] synthesized charge-reversal spherical bridged polysilsesquioxanexerogel (BPS) nanoparticles, which can be triggered by UV irradiation. These featured negative colloidal charges, which are intrinsic to the BPS nanoparticles. Secondary amine groups emerged in the bridging moieties, reversing the nanoparticle charge from negative to positive. It was previously reported that organically derivatized gold nanoparticles functionalized with a photoresponsive linker (thioundecyl-tetraethyleneglycolester-*o*-nitrobenzy-lethyldimethyl ammonium bromide, TUNA) were positively charged with an average particle diameter of 5 nm in PBS (pH = 7.4). The photolabile linker was cleaved with photoirradiation at 356 nm, resulting in the

formation of a cationic compound with negatively charges. The charge repulsion unveiled the mesopores leading to the release of guest molecules[23]. Rotello et al.[24] constructed a positively charged gold nanoparticle joined to a photoactive *o*-nitrobenzyl ester linkage. It could be triggered by light, which allowed a temporal and spatial release of DNA. Near-UV irradiation cleaved the nitrobenzyl linkage, releasing the positively charged alkyl amine and leaving behind a negatively charged carboxylate group. The charge-reversal repulsed DNA from the nanoparticle effectively, resulting a high level of recovery of DNA transcription *in vitro* and *in vivo*.

3.2. *Thermo-responsive charge-reversal drug delivery*

Thermo-responsive drug delivery is among the most investigated stimuli-responsive strategies. Thermo-responsiveness has been widely explored in oncology. Thermo-responsive charge-reversal carriers retain their load at body temperature and reverse their surface charge following thermos-response triggering. Caruso et al.[25] synthesized a poly(allylamine) hydrochloride (PAH) coated SiO_2 nanoparticle. The act of heating could cause the cationic polymer PAH to lose its inherent positive charge and become negative without any polymer desorption. The results of the fouling properties and cell-association behavior of the particles showed that heating reduced the protein fouling and cell association of PAH thin films and particles. Richtering and coworkers[26] constructed magnetic nanoparticles (MNP)-coated carriers, MG/PDADMAC/PSS/MNP (microgels/poly(diallyldimethylammonium chloride)/poly(sodium 4-styrenesulfonate)/magnetic nanoparticles), using layer-by-layer methods. A layer of negatively charged PSS covered the positively charged MNP. MNP was able to generate enough heat to raise the temperature and lead to the collapse of each layer following the charge-reversal from negative to positive.

4. Application of charge-reversal systems in cancer treatment

4.1. *Chemotherapeutics delivery*

A number of anticancer drugs, such as anthracylines, camptothecin and cisplatin, are DNA-toxins. They exert their power through targeting nuclear DNA resulting in DNA damage, or by inhibiting topoisomerase involved in DNA replication to induce cell death (apoptosis)[27,28]. They have to enter the nucleus to elicit their pharmacological responses. However, drug-resistant tumor cells limit the access of cytosolic drugs to the nucleus. P-glycoprotein is overexpressed on the membranes of cytoplasmic organelles and the nuclear envelope in drug-resistant cells. P-glycoprotein activates the intracellular drug sequestration and outwards transport of drugs from their intracellular targets[29].

Charge-reversal nanoparticles switch the negatively charged surface to positively charged surface for the enhanced cellular uptake due to the enhanced nanoparticle-cellular membrane interaction[30–32]. Wang and colleagues[33] developed a kind of charge-reversal nanoparticles based on zwitterionic polymer by introducing a tumor extracellular acidity-sensitive group as the anionic part of the Zwitterionic polymer. The block copolymer of poly(*ε*-caprolactone) (PCL) and poly(allyl ethylene phosphate) (PCL-*b*-PAEP) was well established by controlled ring-opening polymerization. The amphiphilic Zwitterionic block copolymer PCL-*b*-(PAEP-*g*-TMA/DMA) self-assembled and encapsulated DOX. The Zwitterionic polymer diminished its anionic part, forming PCL-*b*-(PAEP-*g*-TMA/Cya) in response to the lower pH. The formed nanoparticles can switch from negative charge to positive charge and become recognizable by tumor cells. Zhang and coworkers[34] developed methoxy poly(ethylene glycol)-*b*-poly (*ε*-caprolactone-*co*-*γ*-dimethyl maleamidic acid-*ε*-caprolactone) [mPEG-*b*-(PCL-*co*-DCL)] for the pH-tailored charge-reversal of intracellular delivery of DOX. The *β*-carboxylic amide-functionalized polymer micelles are negatively charged and regarded stable in neutral solution. They quickly turn positive at pH 6.0 due to the hydrolysis of *β*-carboxylic amides in acidic conditions. The MTT results implied that mPEG-*b*-(PCL-*co*-DCL) micelles were biocompatible with HepG2 cells while DOX-loaded micelles showed significant cytotoxicity. Xing et al.[35] constructed a charge-reversal graphene oxide (GO) for the controlled release of anticancer drugs, such as DOX. Citraconic anhydride-functionalized PAH (PAH-Cit) is a charge-reversal polyelectrolyte, which can convert to poly(allylamine) in the acidic environments, such as endosomes and lysosomes. They developed a GO-based charge-reversal nanocarrier (GO-Abs/PEI/PAH-Cit/Dox) for the enhanced delivery into U87 MG tumor-bearing nude mice. Treatment of this DOX-loaded nanoparticle for 12 h showed strong fluorescence, suggesting an effective DOX delivery by this GO particle.

4.2. *Gene delivery*

Gene therapy has received tremendous attention due to its potential application for delivering missing genes or functional substitutes of defective genes. Effective gene-delivery vectors hold a vital role in the success of gene therapy. These vectors could transport plasmid DNA, small interfering RNA, or antisense oligonucleotides into target cells. Viral and synthetic vectors are the two most common methods for gene delivery. Gene-delivery *via* viral approach is conventional and efficient.

Compared with virus vectors, there are intense studies on nonviral vectors for their low toxicity, high loading capacity, nonimmunogenicity, and ease of synthesis. Yet, limitations, such as low transfection efficiencies and inactivation in the presence of serum, restrict the wide application of nonviral vectors. To overcome these limitations, charge-reversal strategies, such as modifying the amphile structure and adding an alternative cationic head group, have been used for the efficient delivery of genes.

Poly(L-lysine) (PLL)- and polyethylenimine (PEI)-based gene carriers have been widely used as non-viral gene carriers[36]. They can promote the entry of carriers into the nucleus[37]. Drug and cationic polymer conjugates might be delivered into the nucleus. However, the cationic nanoparticles have strong non-specific cellular uptake in the bloodstream that could lead to severe serum inhibition following rapid clearance from the plasma compartment, which limits their function as drug carriers *in vivo*[38]. So an ideal delivery strategy might be masking the positive charge during blood circulation, but reversing it upon arrival at the tumor site.

The charge-reversal nanoparticle mainly performs two roles: first, it releases the genes after delivering them to the tumor site; second, it destabilizes the bilayer and reverses surface charges upon stimulation by the tumor microenvironment or manual intervention (near infrared light, thermo). Liang and coworkers[39] developed a nanocarrier system coated with chitosan and a pH-responsive charge-reversible polymer, PAH-Cit, to deliver siRNA. The citraconic amide side chains of the anionic charge-shifting polymer, PAH-Cit, were hydrolyzed under lower pH conditions

and changed to cationic PAH. It destructed the layer-by-layer structure of the nanoparticles and thus repelled the PEI/siRNA. The amino groups on PAH contributed to the "proton-sponge" effect to facilitate the release of siRNA. This overcame the strong binding between Au nanoparticles and siRNA. Pfeifer et al.[40] synthesized well-defined cationic polylactides (CPLAs) with tertiary amine groups using thiol-ene click functionalization of an allyl-functionalized polylactide to yield polymers with tunable charge densities. Kempson and coworkers[41] developed branched polyethylenimine (bPEI) and copolymers, consisting of PEG, histidine (His), and glutamic acid (Glu). The bPEI25K/siRNA/poly (PEG-His-PEG-Glu) had a hydrodynamic size of 150 nm and negative ζ-potential ($\sim$10.5 mV). Flow cytometry results showed an increased fluorescence intensity in a step-wise manner depending on pH to release siRNA.

4.3. *Protein delivery*

In the last decade, pharmaceutically active peptides and proteins have been invented with the progress in biotechnological techniques and genetic engineering. However, the application of these novel therapeutic biomolecules are limited by their large size, short plasma half-life, high elimination rate (easy to be deteriorated by enzyme and body fluids), inability to pass through the cell membranes, and poor oral bioavailability. This leads to the frequent injection of drug over a long treatment period when such biomolecules are used clinically[42]. The traditional administration methods for protein drugs are oral and parenteral administration. Degrading factors (*e.g.*, water and enzymes) limit the effectiveness of these approaches.

Entrapment of these drugs into a particular carrier offers an effective approach to overcome these problems. The strategy of delivering proteins to biological compartments by nanoparticle is a promising technique to improve protein bioavailability. Polylactic acid (PLA) and its co-polymer with glycolic acid (*i.e.*, PLGA) are accepted by the regulatory authorities for parenteral administration as implants (*e.g.*, Zoladex®) and microparticles (*e.g.*, Decapeptyl®, Parlodel LA® and Enantone Depot®)[43]. The design of a novel protein nanoparticle mainly improves protein targeting and activity *in vivo*. It may (1) promote treatment outcomes with reduced adverse effects; (2) avoid drug resistance given that redundant drug dosing and inexact targeting can stimulate drug resistance under pathological conditions; and (3) overcome drug resistance mechanisms with high persistent local drug concentration[32,44].

Charge-reversal polymeric carriers have emerged as more functional protein carriers. Compared with conventional protein carriers, they have improved properties such as increased stability, modulated site specificity, improved blood circulation stability and stimuli-responsive release[45,46].

Akiyoshi et al.[47] constructed an effective intracellular protein delivery system from self-assembled cationic nanogels. They investigated the interaction of proteins with self-assembled nanogels from cationic cholesteryl group-bearing pullulans ($CHPNH_2$). The cationic nanogels strongly interacted with bovine serum albumin (BSA) which formed monodispersed nanoparticles ($<$50 nm). It was found that the complex internalized into HeLa cells effectively. Kissel and coworkers[48] developed negatively charged nano-carriers consisting of polymer blends of PLGA and poly (styrene-*co*-4-styrene-sulfonate) (PSSS) to release the positively charged protein (*e.g.*, lysozyme). This nanoparticle had a high density of negative charge resulting in improvement of the loading capacity of proteins. Raichur et al.[49] produced a stable hollow microcapsules composed of sodium carboxymethyl cellulose (CMC) and PAH using layer-by-layer techniques to absorb polyelectrolytes onto $CaCO_3$ microparticles. A positively charged protein bovine serum albumin (BSA) was spontaneously loaded below its isoelectric point into hollow microcapsules. In the acidic pH range a fluorescent probe, FITC-dextran, could be loaded, indicating that the shell was permeable. As the pH was increased the permeability decreased. No FITC-dextran was encapsulated when the pH was above 7.0, indicating that the capsule walls were impermeable. At acidic pH, the amino groups of PAH were protonated leading to a local excess of positive charges. These charges repulsed electrostatically resulting in transition from a continuous to a nanoporous morphology of walls of microcapsules. When the pH was increased, the charge of the microcapsule wall reversed, reducing the degree of electrostatic repulsion and leading to compaction of the layers and closing of the pores[50].

5. Conclusions

The design of a drug delivery system often uses a specific ligand for a tumor target. However, less than 5% of the dosage of an intravenous injection is able to reach the tumor site. Smart nanocarriers sensitive to exogenous or endogenous stimuli represent an alternative targeted drug delivery method. A wide range of stimuli is able to trigger drug release at the desired place and time, and the diversity of responsive materials has been assembled in different architectures, allowing great flexibility in the design of stimuli-responsive systems on-demand.

Charge-reversal delivery strategies are designed to be sensitive to specific stimuli, such as a lowered interstitial pH, a higher glutathione concentration, or an increased level of certain enzymes such as MMP. At the cellular level, pH sensitivity can either trigger the release of the transported drug into late endosomes or lysosomes, or promote the escape of the nanocarriers from the lysosomes to the cell cytoplasm. At the tissue level, one can take advantage of specific microenvironmental changes associated with neoplastic diseases (the treatment of which is the focus of most research efforts on stimuli-responsive nanocarriers) as well as pathological situations, such as ischemia, inflammatory diseases or infections. The ability to switch the surface charge allows one to avoid the unspecific absorption and enhance the tumor target delivery.

As discussed in this review, considerable progress in materials chemistry and drug delivery has led to the design of charge-reversal concepts using well-engineered nanosystems. The focus should now shift towards clinically acceptable systems that are more sensitive to discrete variations in specific stimuli. We believe that charge-reversal drug delivery strategies for targeting tumor treatment will provide promising avenues to treat cancers in the future.

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