pH-responsive Nanoparticles for Drug Delivery

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Abstract

First-generation nanoparticles (NPs) have been clinically translated as pharmaceutical drug delivery carriers for their ability to improve on drug tolerability, circulation half-life, and efficacy. Towards the development of the next-generation NPs, researchers have designed novel multifunctional platforms for sustained release, molecular targeting, and environmental responsiveness. This review focuses on environmentally-responsive mechanisms used in NP designs, and highlights the use of pH-responsive NPs in drug delivery. Different organs, tissues, and subcellular compartments – as well as their pathophysiological states – can be characterized by their pH levels and gradients. When exposed to these pH stimuli, pH-responsive NPs respond with physicochemical changes to their material structure and surface characteristics. These include swelling, dissociating or surface charge switching, in a manner that favors drug release at the target site over surrounding tissues. The novel developments described here may revise the classical outlook that NPs are passive delivery vehicles, in favor of responsive, sensing vehicles that use environmental cues to achieve maximal drug potency.

Keywords

nanoparticles; drug delivery; responsive; pH; acid

1. Introduction

In the past decade, a myriad of nanoparticle (NP)-based drug delivery systems have been used for clinical applications that range from oncologic to cardiovascular disease.\textsuperscript{1,2} These nanomedicines improve on existing therapeutic agents through their altered pharmacokinetics and biodistribution profiles. To further improve on NP therapeutic efficacy, researchers have begun to explore the use of environmentally-responsive NPs that can, when exposed to external stimuli, produce physicochemical changes that favor drug release at the target site.\textsuperscript{3} These external stimuli include (i) physical signals such as temperature, electric field, magnetic field, and ultrasound; and (ii) chemical signals such as pH, ionic strength, redox potential, and enzymatic activities. NP systems that include

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Conflict of Interest Dr. Omid C. Farokhzad has financial interest in BIND Biosciences and Selecta Biosciences, biopharmaceutical companies developing therapeutic targeted nanoparticles.
liposomes, polymeric micelles, lipoplexes, and polyplexes have been developed to use these physical and chemical cues to modify drug release properties.4,5

Among these environmental stimuli, pH gradients have been widely used to design novel, responsive NPs. This review assesses pH-responsive NP-based drug delivery at three levels, namely at the level of (i) organs, (ii) tissues, and (iii) within subcellular compartments (Figure 1). In particular, we will take specific examples from oral drug delivery, tumor targeting, and intracellular delivery to highlight conceptually interesting pH-responsive NP designs. At the organ level, NP-based oral delivery systems have been formulated for differential drug uptake along the gastrointestinal (GI) tract (Figure 1a).6,7 At the tissue level, NP formulations have been designed to exploit the pH gradients that exist in tumor microenvironments to achieve high local drug concentrations (Figure 1b).8,9 Finally, at the intracellular level, pH-responsive NPs have been designed to escape acidic endo-lysosomal compartments for cytoplasmic drug release (Figure 1c).10,11

Hence, NP formulations that respond to pH gradients within the microenvironments of organs, tissues, and cell organelles may be useful additions to the spectrum of NP-based vehicles available for therapeutic drug delivery.

2. pH-responsive drug delivery at the organ level: oral drug delivery

Each segment of the gastrointestinal (GI) tract maintains its own characteristic pH level, from the acidic stomach lumen (pH 1–3) for digestion,12 to the alkaline duodenum and ileum (pH 6.6–7.5) for the neutralization of chyme.13, 14 Oral delivery is an attractive drug delivery route for its convenience, patient compliance, and cost-effectiveness. However, orally-delivered drugs are exposed to strong gastric acid and presystemic enzymatic degradation, resulting in poor systemic exposure. Therefore, it has proven to be a challenge to achieve adequate and consistent bioavailability levels for orally-administered drugs.15,16

Until now, NPs formulated with biodegradable polymers have been used to improve bioavailability of easily-degraded peptide drugs such as insulin17,18, calcitonin19, and elctotonin20. More recently, newer nanomedicines have included pH-responsive mechanisms to improve systemic exposure from greater gastric retention, trans-epithelial transport, and cellular targeting with surface-functionalized ligands.21,22

One widely adopted approach to achieve organ-specific drug release is to formulate NPs that exhibit pH-dependent swelling. For example, when acrylic-based polymers such as poly(methacrylic acid) (PMAA) are used, NPs retain a hydrophobic, collapsed state in the stomach due to the protonation of carboxyl groups. After gastric passage, an increase in pH leads to NP swelling due to carboxyl ionization and hydrogen bond breakage. Based on these properties, PMAA–poly(ethylene glycol) (PEG) diblock co-polymers were able to achieve swelling ratios (mass of swollen polymer/mass of dry polymer) of 40–90 fold depending on copolymer composition and PEG graft length.23 When NPs were loaded with insulin, ~90% of the insulin was released at pH 7.4 within two hours in their swollen state, whereas only a small fraction (approximately 10%) of the insulin was released at pH 1.2 in their collapsed state.

In addition, PMAA copolymers that contain other components such as polyethylacrylate (PMAA–PEA) and polymethacrylate (PMAA–PMA) show pH-dependent dissolution that may be tailored to respond to the pH of different intestinal regions.24 For example, Eudragit® L100-55, a commercial formulation of PMAA–PEA, dissolves at pH > 5.5 and is therefore suitable for duodenal drug release. Similarly, Eudragit® S100, a commercial formulation of PMAA-PMA, dissolves at pH > 7.0 and is suitable for ileal drug release.25

Mol Pharm. Author manuscript; available in PMC 2012 June 20.
Researchers have designed NPs that undergo a surface charge reversal after gastric passage, with the hope that drug release will occur in the alkaline intestinal tract instead. Using inorganic materials such as mesoporous silica, NPs were surface-functionalized with different densities of positively-charged trimethylammonium (TA) functional groups. The positively-charged TA facilitated loading and trapping of anionic drugs such as sulfasalazine (an anti-inflammatory prodrug for bowel disease) in acidic environments (pH <3). When the drug-loaded NPs were placed in physiological buffers (pH 7.4), a partial negative surface charge on the NPs was generated from the deprotonation of silanol groups; this electrostatic repulsion triggered the sustained release of loaded molecules.

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pH-responsive NPs have been used to preferentially release drugs at sites of disease. Heparin-chitosan NPs were formulated and applied to treat Helicobacter pylori infections, given that the mucus layer and epithelium of the gastric lumen has a higher pH than the overall acidic environment of the stomach. The NPs maintained their stability in the gastric lumen attributable to electrostatic interactions within the structures. Upon contact with an H. pylori infection along the gastric epithelium (pH ~7.4), the deprotonation of chitosan occurs, which weakens electrostatic interactions and leads to NP collapse and heparin release. In another study, chitosan, together with poly-γ-glutamic acid, tripolyphosphate, and MgSO₄, was used to formulate ‘multi-ion-crosslinked’ NPs. The NPs were used to encapsulate insulin at < pH 6 and release it at higher pH by chitosan deprotonization and NP destabilization.

NPs have been surface-modified with selective targeting ligands for differential retention along the GI tract. The ligands used in these studies are acid-stable and include lectin, small peptides, and vitamins. For example, chitosan, which has been shown to facilitate particle transcytosis across the intestinal epithelium, was used to formulate PMAA–chitosan–PEG NPs. Vitamin B-12, which enhanced NP apical-to-basal transport in Caco-2 cells was surface-functionalized onto dextran NPs for insulin delivery in vivo. In addition, RGD peptides were used to target to β₁ integrins expressed on the apical side of M cells in vitro and in vivo. Novel peptides were selected using in vivo phage display to identify peptides for targeted NP delivery to the M cells and follicle-associated epithelium (FAE) of the intestines.

Hence, the NPs described here show pH-dependent drug release properties, enhanced membrane permeability, and have been modified with selective targeting ligands. These NPs are promising delivery vehicles for differential retention and uptake along the GI tract, and ultimately, they may improve on the efficacy of orally-delivered nanomedicines.

3. pH-responsive mechanisms at the tissue level: tumor targeting

Human tumors have been shown to exhibit acidic pH states that range from 5.7–7.8 (Figure 1b). The acidity of tumor microenvironments is caused in-part by lactic acid accumulation in rapidly growing tumor cells owing to their elevated rates of glucose uptake but reduced rates of oxidative phosphorylation. This persistence of high lactate production by tumors in the presence of oxygen, termed Warburg’s effect, provides growth advantage for tumor cells in vivo. In addition, insufficient blood supply and poor lymphatic drainage which are characteristics of most tumors also contribute to the acidity of tumor microenvironment. Increasingly, researchers have exploited the acidic tumor pH to achieve high local drug concentrations and to minimize overall systemic exposure. NPs have been formulated for pH-dependent drug release by using polymers that change their physical and chemical properties, such as by swelling and solubility, based on local pH levels. Particularly, NPs take these actions by responding to the acidic pH of tumor microenvironments, as opposed to those in oral drug delivery where the elevated pH is often used as trigger.
To achieve NP swelling, Griset et al. cross-linked NPs using acrylate-based hydrophobic polymers with hydroxyl groups that were masked by pH-labile protecting groups (e.g. 2,4,6-trimethoxybenzaldehyde). The NPs were stable at neutral pH, but the protecting group was cleaved and the hydroxyl groups were exposed at mildly acidic pH (~pH 5). This hydrophobic-to-hydrophilic transformation caused the swelling of NPs and subsequent drug release. Paclitaxel release was shown to be minimal at pH 7.4 (< 10%), whereas nearly all of the drugs were released within 24 h at pH 5. These acrylate-based, pH-sensitive NPs were shown to prevent the rapid growth of LLC tumors in C57Bl/6 mice compared to non-responsive NPs or paclitaxel in solution, suggesting that pH-responsive drug release may be beneficial for drug delivery to tumors.

pH-dependent hydrophobic-to-hydrophilic transitions may also be used to control polymer dissolution, in which the polymer matrix collapses for drug release. Wu et al. formulated NPs using PEG-poly(β-amino ester) polymers that have a pK<sub>b</sub> of ~6.5. At pH 6.4–6.8, amine protonation increased polymer solubility and induced a sharp micellization-demicellization transition for drug release. In another study, Criscione et al. showed that self-assembly of poly(amidoamine) dendrimers occurred at physiological pH, followed by drug release from NP dissolution at pH < 6.

Drug molecules have been conjugated to polymer chains via pH-labile cross-linkers for pH-responsive drug release. Recently, Aryal et al. developed cisplatin–polymer conjugated NPs using hydrazone cross-linkers to achieve low pH drug release. Cisplatin release occurred at pH < 6 due to hydrazone hydrolysis as opposed to poly(lactic acid) (PLA) degradation; this later contributed to enhanced cellular cytotoxicity over free cisplatin in vitro. In another study, chromone conjugated to magnetic Fe<sub>3</sub>O<sub>4</sub> NPs via a Schiff-base bond led to a four-fold improvement in chromone release at pH 5 versus at pH 7.4, an improvement in chromone solubility in buffer solutions from 2.5 to 633 μg/mL, and finally, enhanced cytotoxicity in vitro. For dual-drug delivery, Shen et al. formed liposome-like NPs by conjugating camptothecin to short PEG chains via an ester bond, followed by encapsulating doxorubicin, a hydrophilic drug. When loaded with doxorubicin salts (doxorubicin·HCl), rapid release of both doxorubicin and camptothecin occurred at pH < 5 or when an esterase was added. Likewise, Bruyère et al. synthesized a series of orthoester model compounds which had different hydrolysis rates at pH ranging from pH 4.5–7.4. A summary of acid labile linkers used in conjugation chemistry and their hydrolytic products are listed in Table 1.

To increase NP retention in tumors, NPs have been designed to reverse their surface charge from neutral/negative to positive at the tumor site. In one study, quantum dots and adenovirus-based NPs were surface-functionalized with pH-sensitive poly(L-lysine) (PLL). PLL amine groups were conjugated with biotin–PEG and citraconic anhydride (a pH-sensitive primary amine blocker) to generate carboxylate groups. Under acidic conditions (pH < 6.6), the citraconylated amide linkages were cleaved, resulting in the recovery of positively charged amine groups. This surface charge reversal in turn led to enhanced NP uptake and transfection of HeLa cells.

The pH-responsive mechanisms described here draw upon a general phenomenon which is the acidity of tumor microenvironment. Here, NPs maintain stability in circulation and undergo physicochemical changes that favor localized drug release.

4. pH-responsive NPs at the cellular level: intracellular delivery

Following endocytosis, rapid endosomal acidification (~2–3 min) occurs due to a vacuolar proton ATPase-mediated proton influx. As a result, the pH levels of early endosomes, sorting endosomes, and multivesicular bodies drop rapidly to < pH 6.0. The process of
endosomal acidification can be harmful to the therapeutic molecule being delivered, especially for macromolecules such as DNA, small interfering RNA (siRNA), and proteins. However, endosomal acidification may also be used as a trigger for endosomal escape and payload release, a mechanism hypothesized to occur via a 'proton sponge' effect. Here, NPs absorb protons at endosomal pH, leading to an increase in osmotic pressure inside the endosomal compartment, followed by plasma membrane disruption and NP release into the cytoplasm.

pH-sensitive polymers that buffer endosomal compartments have been grafted with other functional segments for intracellular delivery. For example, a NP platform termed Dynamic PolyConjugates (Mirus Bio LLC) has an amphiphatic endosomolytic poly(vinyl ether) backbone composed of butyl and amino vinyl ethers. The NPs were used to conjugate and deliver siRNA through a reversible disulfide linkage, and included functional components such as PEG and targeting ligands. The Dynamic Polyconjugates provided effective knockdown of two endogenous liver genes, apolipoprotein B and peroxisome proliferator-activated receptor alpha (PPARα) in vivo.

Amine-containing monomers have been used in rational syntheses of polymers that buffer pH in endosomes. For example, an amphiphilic and cationic triblock copolymer consisting of monomethoxy PEG, poly(3-caprolactone) and poly(2-aminoethyl ethylene phosphate) (mPEG45–b–PCL100–b–PPEEA12) was designed for endosomal buffering and siRNA delivery. The NPs were found to effectively silence GFP expression in HEK293 cells without significant cytotoxicity. In another study, Tietze et al. developed β-propionamide-cross-linked oligoethyleneimine polymers for siRNA delivery. The siRNA-encapsulated NPs knocked down nuclear Ran expression without corresponding cytotoxicity. Jeong et al. developed reducible poly(amide ethylenimine) (PEI) polymers by addition copolymerization of triethylenetetramine and cystamine bisacrylamide. The reducible PEI NPs were used to deliver siRNA that suppressed VEGF expression in PC3 human prostate cancer cell lines with lower cytotoxicity compared to linear PEI formulations.

Copolymers made from pH-sensitive monomers and nonionic monomers allow fine-tuning of polymer pKₐ for improved endosomal escape. For example, using copolymers made from monomers with different pKₐ (e.g. dimethylaminoethyl methacrylate and nonionic monomer 2-hydroxyethyl methacrylate), it was possible to adjust NP pH sensitivity, DNA encapsulation efficiency, and monomer toxicity to optimize transfection efficiency.

Biodegradable poly(β-amino ester) (PbAE) polymers contains tertiary amines that have been used for pH buffering. A combinatorial family of PbAE compounds were created by parallel-synthesis using amine- and acrylate-terminated monomers in a Michael addition reaction, without the use of specialized monomers or protection steps. In this study, PbAE NPs were shown to undergo rapid dissolution in acidic microenvironments (pH 6.5) which facilitated drug release. NPs based on PbAE have been applied to deliver small molecule drugs, DNA, and siRNA.

Stealth PEG layers that are stable in circulation but are released in endosomes have been used to facilitate NP endosomal escape. While PEG shedding itself does not cause endosomal disruption, it may aid NP escape by reducing steric and electrostatic hindrance from the PEG layer. Several PEG-sheddable NP formulations have been developed where PEG is grafted onto NPs via pH labile cross-linkers. In these studies, PEG shedding was shown to both favor drug release and gene expression, suggesting a general application for PEG-shedding strategies.

Finally, NP designs may contain protein transduction domains (PTDs), which are cationic, 10–30 amino acid sequences hypothesized to disrupt endosomal membranes upon
endosomal acidification.\textsuperscript{78} The mechanism of PTD membrane penetration is an active research topic and PTDs have been widely used to improve intracellular delivery in oncologic-based applications.\textsuperscript{79–81} In one study, the co-administration of a free tumor-penetrating peptide (e.g. iRGD sequence) was shown to enhance the efficacy of doxorubicin (doxorubicin liposomes), paclitaxel (nab-paclitaxel), and monoclonal antibody (trastuzumab) treatments.\textsuperscript{82}

However, caution must be taken when targeting is used to improve intracellular delivery, because the extent of endosomal acidification is influenced by the choice of targeting ligand used and hence the endocytic pathway taken. For example, surface-modification with folate was shown to lead to endocytosis through recycling centers characterized by near neutral pH of pH 6–7, which may make it less suitable for pH-based mechanisms.\textsuperscript{83}

Hence, pH-sensitive mechanisms are also important at the stages after NPs are internalized, particularly for the release of a payload into the cytoplasm of the target cell. These mechanisms are even more crucial for payloads such as siRNA, DNA, and proteins, where denaturation in the acidic lysosomal compartment may result in a significant drop in efficacy.

5. Concluding Remarks

Novel approaches in pH-responsive NP design and engineering have resulted in improved drug delivery in pre-clinical studies. In this paper, we have reviewed recent progress made in the research and development of pH-responsive NPs for drug delivery at three levels: at the organ, tissue and subcellular levels. The mechanisms employed in these studies are briefly summarized below in Table 2. With sustained effort in tailoring NPs for environmentally-sensitive drug delivery, it is expected that environmentally-responsive approaches will result in next-generation nanomedicines that have extensive medical applications.

Acknowledgments

Authors’ research was supported by National Institutes of Health Grants CA119349 and EB003647 and Koch–Prostate Cancer Foundation Award in Nanotherapeutics.

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\textit{Mol Pharm.} Author manuscript; available in PMC 2012 June 20.


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Figure 1.
Design of acid-responsive NPs for selective drug release. (a) Targeting at the organ level: the GI tract is characterized by a pH gradient. (b) Targeting at the tissue level: solid tumors have a characteristic acidic extracellular environment different from healthy tissues. (c) Targeting at the cellular level: endo-lysosomes are more acidic in comparison to the cytoplasm (shown in red).
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Table 2

Summary of pH-responsive mechanisms used in nanoparticle designs.

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