Expert Opinion on Drug Delivery

ISSN: 1742-5247 (Print) 1744-7593 (Online) Journal homepage: https://www.tandfonline.com/loi/iedd20

Bioresponsive functional nanogels as an emerging platform for cancer therapy

Dechun Huang, Hongliang Qian, Haishi Qiao, Wei Chen, Jan Feijen & Zhiyuan Zhong

To cite this article: Dechun Huang, Hongliang Qian, Haishi Qiao, Wei Chen, Jan Feijen & Zhiyuan Zhong (2018) Bioresponsive functional nanogels as an emerging platform for cancer therapy, Expert Opinion on Drug Delivery, 15:7, 703-716, DOI: 10.1080/17425247.2018.1497607

To link to this article: https://doi.org/10.1080/17425247.2018.1497607

Published online: 16 Jul 2018.

EXPERT OPINION ON DRUG DELIVERY
2018, VOL. 15, NO. 7, 703–716
https://doi.org/10.1080/17425247.2018.1497607

Taylor & Francis
Taylor & Francis Group

REVIEW

Bioresponsive functional nanogels as an emerging platform for cancer therapy

Dechun Huang[a], Hongliang Qian[a], Haishi Qiao[a], Wei Chen[a], Jan Feijen[b,c] and Zhiyuan Zhong[b]

[a]Department of Pharmaceutical Engineering, School of Engineering, China Pharmaceutical University, Nanjing, P. R. China; [b]Biomedical Polymers Laboratory, Jiangsu Key Laboratory of Advanced Functional Polymer Design and ApplicationCollege of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, P. R. China; [c]Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, Netherlands

ABSTRACT

Introduction: Bioresponsive nanogels with a crosslinked three-dimensional structure and an aqueous environment that undergo physical or chemical changes including swelling and dissociation in response to biological signals such as mild acidity, hyperthermia, enzymes, reducing agents, reactive oxygen species (ROS), and adenosine-5′-triphosphate (ATP) present in tumor microenvironments or inside cancer cells have emerged as an appealing platform for targeted drug delivery and cancer therapy.

Areas covered: This review highlights recent designs and development of bioresponsive nanogels for facile loading and triggered release of chemotherapeutics and biotherapeutics. The *in vitro* and *in vivo* antitumor performances of drug-loaded nanogels are discussed.

Expert opinion: Bioresponsive nanogels with an excellent stability and safety profile as well as fast response to biological signals are unique systems that mediate efficient and site-specific delivery of anticancer drugs, in particular macromolecular drugs like proteins, siRNA and DNA, leading to significantly enhanced tumor therapy compared with the non-responsive counterparts. Future research has to be directed to the development of simple, tumor-targeted and bioresponsive multifunctional nanogels, which can be either constructed from natural polymers with intrinsic targeting ability or functionalized with targeting ligands. We anticipate that rationally designed nanotherapeutics based on bioresponsive nanogels will become available for future clinical cancer treatment.

Abbreviations: AIE, aggregation-induced emission; ATP, adenosine-5′-triphosphate; ATRP, atom transfer radical polymerization; BSA, bovine serum albumin; CBA, cystamine bisacrylamide; CC, Cytochrome C; CDDP, cisplatin; CT, computed tomography; DC, dendritic cell; DiI, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; DOX, doxorubicin; dPG, dendritic polyglycerol; DTT, dithiothreitol; EAMA, 2-(N,N-diethylamino)ethyl methacrylate; EPR, enhanced permeability and retention; GrB, granzyme B; GSH, glutathione tripeptide; HA, hyaluronic acid; HAase, hyaluronidases; HCPT, 10-Hydroxycamptothecin; HEP, heparin; HPMC, hydroxypropylmethylcellulose; LBL, layer-by-layer; MTX, methotrexate; NCA, N-carboxyanhydride; OVA, ovalbumin; PAH, poly(allyl amine hydrochloride); PBA, phenylboronic acid; PCL, polycaprolactone; PDEAEMA, poly(2-diethylaminoethyl methacrylate); PDGF, platelet derived growth factor; PDPA, poly(2-(diisopropylamino)ethyl methacrylate); PDS, pyridyldisulfide; PEG, poly(ethylene glycol); PEGMA, polyethyleneglycol methacrylate; PEI, polyethyleneimine; PHEA, poly(hydroxyethyl acrylate); PHEMA, poly(2-(hydroxyethyl) methacrylate; PNIPAM, poly(*N*-isopropylacrylamide); PMAA, poly(methacrylic acid); PPDSMA, poly(2-(pyridyldisulfide)ethyl methacrylate); PTX, paclitaxel; PVA, poly(vinyl alcohol); QD, quantum dot; RAFT, reversible addition-fragmentation chain transfer; RGD, Arg-Gly-Asp peptide; ROP, ring-opening polymerization; ROS, reactive oxygen species; TMZ, temozolomide; TRAIL, tumor necrosis factor-related apoptosis inducing ligand; VEGF, vascular endothelial growth factor.

ARTICLE HISTORY
Received 12 April 2018
Accepted 2 July 2018

KEYWORDS
Nanogels; stimuli-sensitive; drug delivery; tumor microenvironment; anticancer drug; cancer therapy

1. Introduction

Nanogels are nano-sized three-dimensional (3D) hydrogels that are made of physically or chemically crosslinked water-soluble polymers [1]. Nanogels with a high water content present several unique features such as a high porosity, mechanical properties similar to those of the natural extracellular matrix (ECM), good biocompatibility, and excellent compatibility with different bioactives including proteins and nucleic acids [2–6]. In the past years, many efforts have been directed to the development of functional nanogels for enhanced cancer therapy [7–9]. In particular, bioresponsive nanogels that undergo physical or chemical changes including swelling and dissociation in response to biological signals such as enzymes, hyperthermia, mild acidity, reducing agents, reactive oxygen species (ROS), and adenosine-5′-triphosphate (ATP), either present in the tumor microenvironment or inside the cancer cells, are very appealing in that (i) they show site-specific and markedly enhanced drug release at the site of action, boosting anticancer effects; (ii) they are easily applied and less expensive as compared to systems based on external stimuli (e.g. magnetic,

CONTACT Wei Chen None w.chen@cpu.edu.cn Department of Pharmaceutical Engineering, School of Engineering, China Pharmaceutical University, Nanjing P. R. China; Zhiyuan Zhong zyzhong@suda.edu.cn Biomedical Polymers Laboratory, Jiangsu Key Laboratory of Advanced Functional Polymer Design and ApplicationCollege of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou, P. R. China

Article highlights

- Introduction of rational designs, fabrication methods, and unique features of bioresponsive functional nanogels as advanced drug delivery systems.
- Recent designs and development of bioresponsive as well as dual-bioresponsive functional nanogels for triggered release of chemotherapeutics and biotherapeutics including proteins and nucleic acids.
- Novel fabrication methods of cancer-specific and bioresponsive multifunctional nanogels for targeted treatment of various solid tumors and blood cancers.
- Personal opinions on the future development of bioresponsive functional nanogels for cancer therapy.

This box summarizes key points contained in the article.

ultrasonic, near-infrared, temperature) as no external equipment is required to trigger drug release; and (iii) they have excellent patient compliance [10–12]. Notably, bioresponsive nanogels exhibit typically swift responses, which are significantly faster than usually observed for other nanosystems such as micelles, vesicles, and nanoparticles, due to the fact that they have an aqueous internal environment and microporous structure. Furthermore, nanogels are able to stably encapsulate multi-compounds with high loading efficiency and preserve drug bioactivity, which is especially important for peptides/proteins and nucleic acids, playing critical roles in combination therapies [13–15].

Bioresponsive nanogels are usually made by crosslinking functional derivatives of water-soluble polymers that can be of natural or synthetic origin or by employing functional crosslinkers in combination with water-soluble monomers or polymers. Both fabrication techniques and chemistry play a significant role in development of bioresponsive functional nanogels. Techniques like inverse mini-emulsion, inverse nanoprecipitation, microfluidics, and in-situ self-assembly are commonly used for the nanogel fabrication with sophisticated control over size and shape [16–18]. For example, Bazban-Shotorbani et al. applied droplet microfluidics using a 2D hydrodynamic flow focusing method (2D-HFF) to fabricate alginate nanogels with average sizes ranging from 68 to 138 nm, showing a good degree of controllability over the nanogel sizes and their composition [19]. The process of nanogel formation takes place among the polymer chains during either polymerization of low molecular weight monomers using heterogeneous polymerization reactions in the presence of functional crosslinkers, or crosslinking of polymer precursors via a variety of crosslinking approaches including click chemistry, Schiff-base reactions, thiol-disulfide exchange, and photo-initiated crosslinking [20,21]. In particular, the recent developments in click chemistry such as strain-promoted azide-alkyne cycloaddition, Michael-type addition reaction, Diels-Alder reaction, and tetrazole-alkene photo-click chemistry allow the preparation of nano/microgels without the use of potentially toxic catalysts or the interference with encapsulated bioactives owing to its unique bio-orthogonal features [22]. For example, Steinhilber et al. developed pH-degradable hyperbranched polyglycerol (HPG) nano/microgels via the 'copper-free' azide-alkyne cycloaddition reaction for protein and living cell delivery [23,24]. Chen et al. developed reducible hyaluronic acid (HA)-based nanogels with intrinsic fluorescence by combining the inverse nanoprecipitation method and tetrazole-alkene photo-click chemistry for protein delivery and cell imaging [25,26].

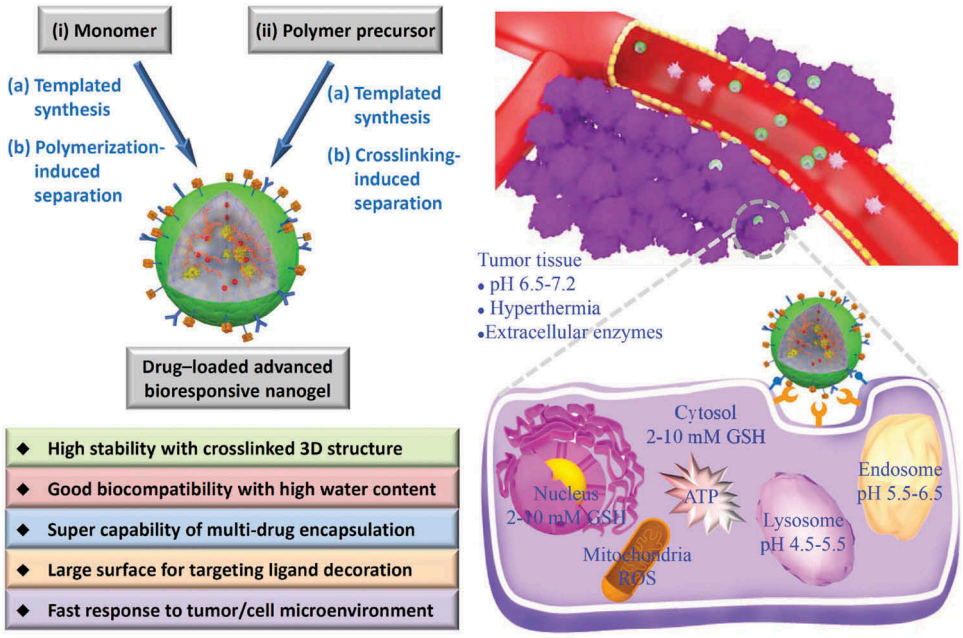
Nevertheless, current nanogel-based drug formulations have to be further optimized to meet the clinical requirements [27]. Bioresponsive nanogels with unique design, especially the chemically crosslinked ones, are highly attractive for targeted drug delivery because of their superior stability under normal conditions but fast response to specific tumor environments, which well solves the problem of high stability for long circulation versus fast drug release at the tumor site. However, another critical challenge for nanogel formulations is their poor tumor accumulation and intratumoral penetration due to the stiff tumor ECM, high interstitial fluid pressure (IFP) as well as their specific affinity for the tumor cells in the tumor microenvironment. Even though some passive tumor-targeting of nanogel systems takes place, their stealth surface (e.g. by PEG and dextran) required for prolonged circulation also results in modest and inefficient cellular uptake, which is detrimental for the effective transport of many anticancer drugs incorporated in nanogels including PTX and DOX into the tumor cells. The advances in polymer chemistry to explore nanogels with controllable composition, architecture, and surface functionality with a specific tumor-homing ligand such as a polysaccharide, antibody, peptide, saccharide or folic acid may lead to improvements with respect to tumor retention and accumulation of nanogels, and selective and efficient internalization by target tumor cells [28–31]. Moreover, dual ligand modification of nanogels may lead to both improved transport to the tumors, improved tumor penetration and uptake by tumor cells. In this review, we will highlight the design rationales as well as the recent exciting progress of bioresponsive nanogels, which are responsive to signals provided in the tumor environment or inside cancer cells to trigger drug release (Scheme 1). We will focus especially on attempts to further fine-tune drug release and augment therapeutic efficacy of nanogel drug formulations. Up to now, tumor targeted bioresponsive nanogels have been explored to achieve improved tumor accumulation and cellular uptake, and unprecedented control over drug release, leading to superior *in vitro* and/or *in vivo* treatment efficacy.

2. pH-responsive nanogels

Lots of efforts have been directed to the development of pH-responsive nanogels for tumor targeted drug delivery, since there are natural pH gradients in the tumor tissue microenvironments (pH 6.5–7.2) as well as in the endosomal/lysosomal compartments in the cells (pH 5.0–6.5) compared with the pH value of normal tissues (pH 7.4) [32]. The strategy of pH-triggered drug release has been explored using various approaches to overcome the various extra/intra-cellular barriers for drug delivery and to develop a successful anticancer therapy.

2.1. Amino-based pH-responsive nanogels

In the past several years, pH-responsive nanogels containing protonable amino groups, such as primary, secondary and



Scheme 1. Illustration of fabrication and unique features of bioresposive functional nanogels, which respond to biosignals such as enzymes, hyperthermia, mild acidity, reducing agents, ROS, and ATP, either present in the tumor microenvironment or inside the cancer cells.

tertiary amines, have been designed for targeted drug delivery, since they are prone to swelling at mildly acidic pHs to trigger drug release [33–35]. For example, Bae's group synthesized a virus-mimetic nanogel (VM-nanogel) system with a hydrophobic core made of poly(L-histidine-co-phenylalanine) (poly(His_{32}-co-Phe_6)) and a two-layer hydrophilic shell containing PEG as an inner shell and bovine serum albumin (BSA) as a capsid-like outer shell [36]. The swelling of the nanogel core at endosomal pH (pH 6.4) enhanced the release of DOX, whereas the nanogel expansion combined with the proton buffering effect of polyHis facilitated endosomal escape. DOX-loaded VM-nanogels efficiently killed A2780/AD cells by intracellular DOX release, and could further migrate to neighboring tumor cells to exert their bio-functions. pH-responsive nanogels have been fabricated from natural chitosan and gelatin to achieve controlled release of drugs for anticancer treatment [15]. Zhang et al. also explored a virus-like nanogel system based on poly(*N*-isopropylacrylamide) (PNIPAM) and *N*-lysinal-*N'*-succinyl modified chitosan (NLSC) with a BSA capsid-like shell for deep tumor penetration by pH-induced reversible swelling-shrinking [37]. Like the virus invasion, these nanogels migrated from one dead HepG2 cell to the neighboring cells for repeating efficacy (Figure 1). Song et al. prepared a biomimetic nanogel composed of hydroxypropyl-β-cyclodextrin acrylate and two oppositely charged chitosan derivatives for combinatorial chemotherapy and immunotherapy [38]. The nanogel shielded with an erythrocyte membrane was able to deliver the immunotherapeutic agent interleukin-2 via the so-called 'nanosponge' properties, followed by precisely pH-controlled release of the anticancer drug paclitaxel in the tumor microenvironment, significantly enhancing the antitumor activity with improved drug penetration and increased antitumor immunity.

pH-sensitive nanogels are emerging as promising carriers for treating skin cancer via the transdermal route, which has shown numerous beneficial properties such as improving patient compliance since they are conveniently dosed compared to intravenous and oral therapy [39]. Curcumin-loaded chitin nanogels with a good and stable colloidal dispersion in water showed a pH-dependent release profile for transdermal drug delivery [40]. A375 melanoma cells showed higher cellular uptake of curcumin-loaded chitin nanogels compared to human dermal fibroblasts (HDFs), resulting in higher toxicity for A 375 melanoma cells. *Ex vivo* studies carried out using porcine skin showed 4-fold higher penetration for curcumin-loaded nanogels in comparison to a curcumin solution, in which the nanogels were more located in the deeper layers of the epidermis and dermis. Histopathological evaluation of the skin clearly indicated the loosening as well as the fragmentation of the stratum corneum in case of the nanogel-treated groups. Divya et al. used chitin nanogels to encapsulate two anti-psoriatic drugs, acitretin (Act) and aloe-emodin (AE) by simple regeneration chemistry [41]. The systems exhibited higher swelling and release at acidic pH. The *ex vivo* skin permeation studies using porcine skin confirmed the higher deposition of the systems at epidermal and dermal layers. Further *in vivo* anti-psoriatic activity study using Perry's mouse tail model and skin safety studies showed potential benefits for topical delivery of Act and AE in psoriasis.

Recently, Gao et al. developed a pH-sensitive nanogel system based on gelatin coated by mesenchymal stem cell membranes for high tumor targeting and therapy [42]. The coating of stem cell membranes on the nanogels surface facilitated a remarkable stability and tumor-targeting toward HeLa cells, followed by endosomal pH-triggered DOX release, resulting in significant antitumor efficiency in a human ovarian cancer model.

The amino-containing nanogels owing to their pH dependent positive charge can not only be used for the

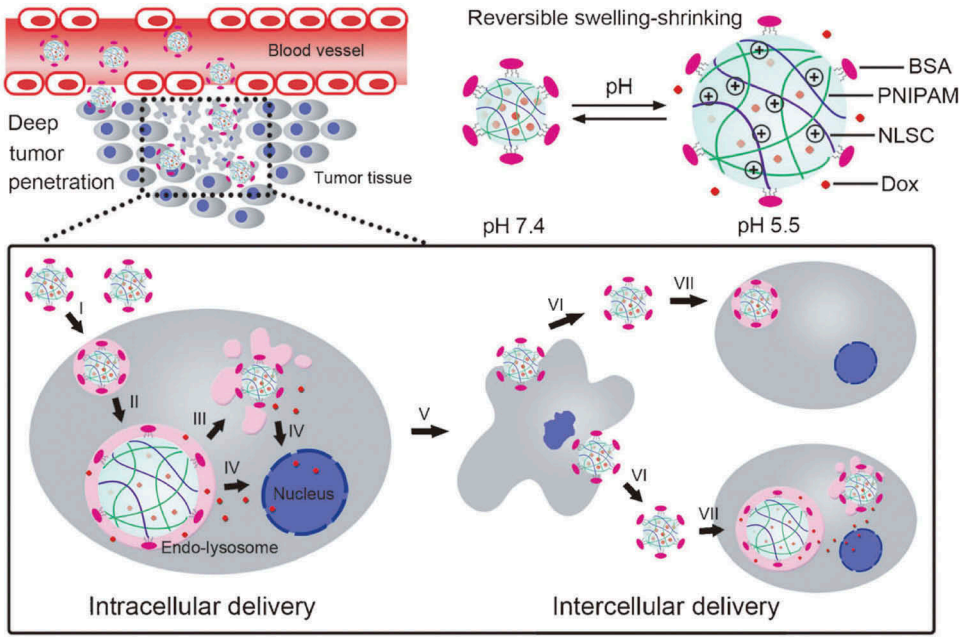


Figure 1. Schematic illustration of an intra-intercellular delivery system based on a reversible swelling–shrinking nanogel for deep tumor penetration. Reproduced with permission from Wiley [37]

encapsulation of small drugs, but also for the incorporation of proteins or nucleic peptides, which can be subsequently delivered into cells. Khaled et al. developed hybrid silica nanogels shielded with crosslinked cationic poly(2-diethylaminoethyl methacrylate) (PDEAEMA) for intracellular delivery of siRNA [35]. The protonation of the tertiary amine in the shell at acidic pH facilitated endolysosomal escape and siRNA release, and also caused efficient silencing of the CXCR4 expression in an orthotopic human breast cancer-bearing mouse model due to tumor accumulation followed by uptake and endosomal escape of the nanogels. Guével et al. prepared a self-assembled hybrid gold nanocluster (Au NCs) nanogels loaded with the 21 amino acid consisting peptide (TRALNNVNRIGNCCAPPVAGG) and the Donkey IgG antibody (*Ab*) for combination of efficient fluorescence imaging and enhanced intracellular protein delivery [43]. The cationic poly (allyl amine hydrochloride) (PAH) coated on the Au nanoparticles exhibited reversible swelling-shrinking properties in the pH range of 6 to 10, directly controlling the aggregation-induced emission (AIE) phenomenon.

2.2. Carboxyl-based ph-responsive nanogels

Carboxyl-containing nanogels also exhibited pH-sensitive drug release due to the decreased electrostatic interactions with the cargoes at acidic conditions. For example, Li et al. developed a core-shell microsphere composed of poly(methacrylic acid) (PMAA) nanogels as the core and a chitosan/alginate (CS/AL) multilayer as the shell via layer-by-layer (LBL) assembly [44]. Compared to the bare PMAA nanogels, the core-shell microspheres displayed a higher DOX loading and an improved pH-controllable drug release. Lin et al. used natural lysozyme and pectin to fabricate biodegradable nanogels for MTX delivery, in which MTX release was accelerated at acidic pH. These nanogels induced enhanced apoptosis of HepG2 cells as compared with free MTX [45]. Su et al. developed thermo-/pH-responsive poly(N-isopropyl acrylamide-co-acrylic acid) nanogels functionalized with iRGD-conjugated gold nanoclusters (AuNCs) on the surface for targeted drug delivery [46]. DOX trapped in the negatively charged swollen NGs could be released in a controlled way. The cellular uptake of the NGs was enhanced by the use of the iRGD motif and the intracellular tracking was facilitated by the AuNCs both in HUVECs and B16 cells. The confocal laser scanning microscopy revealed that the majority of the nanogels were internalized into the cells and located at lysosomes, leading to a desirable pH-dependent fast drug release. Wu et al. developed pH-responsive nanogels based on hydroxypropylcellulose-poly (acrylic acid) (HPC-PAA) with immobilized CdSe quantum dots (QDs) [47]. Hydrophilic temozolomide (TMZ) drug was efficiently loaded into the hybrid nanogels and exhibited an acidic pH-triggered release profile. The hybrid nanogels can also be used for pH-sensing with a strong NIR emission at 741 nm for detecting the physiochemical environment (pH 7.4) and a less sensitive visible emission at 592 nm for imaging mouse melanoma B16F10 cells [48]. They also designed another hybrid nanogel system based on covalently cross-linked poly(methacrylic acid) (PMAA) networks semi-interpenetrated with chitosan chains to simultaneously facilitate biosensing, bioimaging, and pH-regulated delivery of TMZ.

2.3. Acid-labile bond-based pH-responsive nanogels

Acid-labile bonds including hydrazine, cis-aconityl and acetal/ketal groups in nanogel systems can degrade fast at mild acid pH conditions, which facilitates either efficient cellular uptake of nanogels or rapid dissociation of the gels for intracellular drug release. For example, Du et al. fabricated ultra pH-responsive

nanogels containing 2,3-dimethylmaleic imide bonds which could efficiently degrade at the extracellular tumor pH (pH 6.8) to convert the surface charge from negative to positive for enhanced cellular uptake, followed by pH-triggered intracellular DOX release in MDA-MB-435s cells [49]. The negative control consisting of a non-charge-conversional nanogel was only located in the extracellular space or on the cell membrane. Chen et al reported that pH-degradable PVA nanogels based on vinyl ether acryl (VEA) functionalized PVA were fabricated with tailored nanogel sizes by photo-crosslinking of thermo-induced nanoaggregates [50]. These nanogels showed pH-triggered PTX release and fast acid-degradation into native PVA, poly(hydroxyethyl acrylate) (PHEA) and acetaldehyde. Li et al. developed pH-responsive nanogels based on a block copolymer of PEG-b-poly[*N*-[*N*-(2-aminoethyl)-2-aminoethyl]-L-glutamate] (PEG-b-PNLG) by the formation of benzoic imine bonds using terephthalaldehyde (TPA) as a cross-linker [51]. The cleavage of benzoic imine bonds in tumoral acidic environments (pH ~6.4) induced the destruction of nanogels and resulted in rapid release of their payloads. The DOX-loaded nanogels exhibited higher cytotoxicity against the breast cancer cell line MDA-MB-231 than free DOX, with IC_{50} values of DOX-loaded nanogels and free DOX of 3.66 and 15.45 μg/mL, respectively.

pH-Degradable nanogels are also highly promising for protein delivery. For example, Steinhilber et al. designed acetal-degradable nanogels based on dendritic polyglycerol (dPG) via a bio-orthogonal 'click' reaction by an inverse nanoprecipitation procedure [24]. The enzyme activity and structural integrity of the model protein asparaginase were retained after encapsulation and acid-triggered release. Recently, Dimde et al. devised a pH-sensitive nanogel platform based on dPG and low molecular weight polyethylenimine units for siRNA delivery [52]. These cationic nanogels were able to encapsulate siRNA. The pH-sensitive benzacetal-bonds enabled the controlled intracellular release of siRNA, which significantly improved silencing of the GFP expression in HeLa cells as compared to non-degradable PEI controls.

3. Enzyme-responsive nanogels

Enzymes are known to be not only efficient but also highly specific catalysts for chemical transformations under aqueous conditions. In the tumor microenvironment and lysosomes, there are abundant enzymes such as proteolytic enzymes, hyaluronidase, lipase, matrix metalloproteinases (MMP) and lysosomal enzymes. In the past years, various enzyme-responsive nanogels have been designed to obtain triggered release of drugs and proteins in the tumor extracellular environment or in cancer cells [53–55]. It is interesting to note that enzymes in the tumor environment can not only trigger drug release but also improve tumor penetration and cellular uptake. Hyaluronic acid (HA) is a natural material with intrinsic targetability toward CD44-overexpressed tumor cells. The degradation of HA can be triggered by extracellular overexpressed hyaluronidases (HAase) in tumors. Yang et al. developed HA nanogels via radical copolymerization of methacrylated HA and di (ethylene glycol) diacrylate (DEGDA) in aqueous solution for CD44-targeted delivery of DOX [56]. Rapid dissociation of HA nanogels was triggered by the combination of lipase and hyaluronidase, resulting in enhanced DOX accumulation at the tumor site and significant tumor inhibition. Jiang et al. developed a programmed drug-delivery nanogel-liposome system composed of a liposomal core and a crosslinked HA shell for the sequential and site-specific delivery of tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and DOX [57]. The rapid degradation of the HA shell by HAase in the tumor environment induced fast extracellular release of TRAIL and subsequent internalization of the liposomes. The IC_{50} of TRAIL and DOX co-loaded system was 83 ng mL^{-1} toward MDA-MB-231 human breast cancer cells, which was 5.9-fold higher than that of the DOX only (single drug) system. More recently, Zhu et al. developed HAase-degradable HA nanogels crosslinked by radical polymerization of self-assembled cholesteryl methacrylated HA. These nanogels were used for systemic delivery of the anticancer protein deoxyribonuclease I and efficient therapy of lung cancer [58]. The anticancer protein and citraconic anhydride shielded HAase were co-encapsulated in nanogels, in which HAase could be activated by hydrolysis of the citraconic amide under acidic condition, resulting in fast enzymatic degradation of HA nanogels and accelerated protein release, both in the acidic tumor microenvironment and intracellularly. *In vivo* therapeutic efficacy studies using A549 tumor-bearing mice showed that the anticancer protein and shielded HAase co-loaded nanogels had a more pronnouced tumor-inhibiting effect than the nanogels only loaded with the anticancer protein (tumor inhibitory ratio: 62% *vs* 41%), which was mostly attributed to enhanced tumor penetration and protein release by the tumor-specific activation of nanogel self-destruction.

Wang et al. prepared G4-PAMAM dendrimer-based nanogels containing the bioadhesive RGDC peptide and enzyme-responsive RAADyC peptide (arg-ala-ala-asp-D-tyr-cys-NH2) for targeted DOX delivery [59]. The size of the nanogels decreased fast in the presence of elastase with sustained DOX release, which was due to the specific cleavage of RAADyC peptide and peeling off the nanogel by elastase. Xiong et al. reported bacteria-sensitive triple-layered nanogels (TLN) that responded to a special bacteria-accumulated tumor environment for differential anticancer drug delivery within a tumor [60]. DOX-loaded TLN (TLND) was selectively degraded in the attenuated SBY1 bacteria-accumulated tumor environment, facilitating differential DOX release and selective killing of tumor cells. Recently, Wang et al. designed and synthesized enzyme-responsive nanogels by free radical precipitation polymerization using *N*-isopropylacrylamide as the scaffold and bis-acryl functionalized MMP2-responsive peptide (AAc-GPLGVRGK-AAc) as the crosslinker. These nanogels were used for hydrophilic P-5m peptide encapsulation and tumor metastasis inhibition [61]. Nanogels with narrow-distributed sizes exhibited a high P-5m loading capacity of 43.4% and excellent and specific MMP2-triggered payload release properties. The nanogels suppressed lung cancer cell migration and invasion, ultimately achieving remarkable tumor metastasis inhibition *in vivo*.

4. Thermosensitive nanogels

The distinct hyperpyrexia locally in malignant tumors is always used as a bio-signal to induce the changes of thermo-sensitive nanogels in their particle volume, which is conveniently

applied to control drug release from these nanogels. Nanogels based on PNIPAM and its copolymers exhibit good thermo-sensitivity with a lower critical solution temperature (LCST) of around 32°C. For example, thermo-sensitive nanogels composed of chitosan (CTS) grafted with poly(NIPAM-co-acrylamide) through free radical polymerization were optimized with a volume phase transition temperature of 38°C by adjusting the ratio of NIPAM/AAm at 5.5 wt.% [62]. Because of electrostatic absorptive endocytosis, thermo-responsive nanogels showed enhanced cellular uptake after loading with coumarin-6, and nanogels encapsulated with PTX exhibited noticeable antitumor efficacy in the human colon carcinoma HT-29 cells xenograft nude mice model. Molina et al. developed thermo-sensitive PNIPAM-HPG nanogels with positive or negative charges by semi-interpenetration and *in situ* polymerization of the corresponding pH-sensitive monomers of (2-dimethylamino) ethyl methacrylate (DMAEM) or 2-acrylamido-2-methylpropane sulfonic acid (AMPS) into PNIPAM-HPG nanogels to modulate the encapsulation and the release of DOX, resulting in overcoming drug resistance in cancer cells [63]. The *in vivo* study showed that free DOX had a moderate inhibitory effect on the growth of the DOX-resistant MaTu/ADR tumor with an inhibition of about 27%, while DOX-loaded thermo-sensitive semi-interpenetrated nanogels caused a significant tumor growth inhibition up to 75%.

Shirakura et al. developed thermo-sensitive nanogels based on the combination of acrylic acid and acrylamide for controlled delivery of cisplatin (CDDP) [64]. It was interesting that with the combination of acrylamide and acrylic acid a UCST like system could be constructed based on the cleavage of hydrogen bonds between acrylic acid and acrylamide at elevated temperature, causing swelling of the nanogel matrix rather than de-swelling. *In vitro* cytotoxicity experiments confirmed that CDDP-nanogels had a remarkably greater efficacy at slightly higher temperatures.

5. Reduction-sensitive nanogels

The concentration level of glutathione tripeptide (GSH) is about 2–3 orders higher (approximately 2–10 mM) in the cytosol and nuclei than in the extracellular fluids (approximately 2–20 μM) [65,66]. Furthermore, the GSH concentration in some tumor tissues is about 4-fold higher than that in normal tissues. It was also reported that a substantial amount of γ-interferon-inducible lysosomal thiol-reductase (GILT) is present in endosomes and lysosomes, which could cleave disulfide bonds at low pH [67]. This significant difference in GSH levels has led to the design of redox-responsive nanogels containing disulfide bonds which can be disassembled in the cytosol and nuclei of cells. The disulfide bonds can be introduced either into the polymer chains or in the crosslinkers, using disulfide containing molecules (e.g. lipoic acid and cystamine) or thiol-disulfide exchange reactions [68–70].

Thayumanavan's group developed reducible nanogels by the self-assembly of random copolymer with 30% of hydrophilic oligo(ethylene glycol)methacrylate and 70% of lipophilic pyridyldisulfide (PDS)-bearing methacrylate monomer, followed by crosslinking via the dithiothreitol (DTT)-initiated thiol-disulfide exchange reaction [71]. The residual PDS functionalities in the nanogels provided useful handles to introduce thiolated ligands like TAT peptide and FITC on the nanogel surface for cell targeting and imaging. The hydrophobic guest molecules encapsulated in the nanogels were efficiently released in the presence of 10 mM GSH, while almost no release was observed with 10 μM GSH, mimicking the GSH concentration outside the cell and within blood plasma. More recently, Park and Kim et al. reported a self-crosslinked reducible HPG nanogel synthesized by anionic ring-opening polymerization of 2-((2-(oxiran-2-ylmethoxy) ethyl)disulfanyl) ethan-1-ol (SSG) monomer, followed by thiol-disulfide exchange reaction with a catalytic amount of DTT [72]. The nanogels could not only retain small molecular therapeutics irrespective of their hydrophilic or hydrophobic nature but also large enzymes during the self-crosslinking chemistry, and these nanogels could further accomplish the controlled release of active therapeutic agents under the reducible cytosol conditions for cancer therapy.

Zhong et al. developed reducible nanogels with a hydrodynamic size of 152–219 nm via self-assembly of HA-lysine-lipoic acid conjugates followed by DTT-catalyzed self-crosslinking [73]. These reducible HA nanogels mediated active targeting delivery and fast release of DOX to CD44-positive breast cancers *in vivo*, effectively overcoming drug resistance (ADR) and prolonging mice survival rate. Notably, DOX-loaded the reducible HA nanogels induced also highly efficacious and targeted inhibition of human hematological cancer xenografts in nude mice such as LP-1 human multiple myeloma (MM) and AML-2 human acute myeloid leukemia [74]. To further improve drug loading capability and reduce nanogel sizes, lipoic acid was linked to HA via poly(γ-benzyl-L-glutamate) (PBLG) [75]. These nanogels exhibited a high DOX loading ability of 25.8 wt.% and small size of 72–80 nm due to presence of π-π interactions between PBLG and DOX. These compacted HA nanogels showed little drug leakage under physiological conditions while quickly releasing ca. 92% DOX in 30 h in a cytosol-mimicking reductive environment. The *in vivo* studies showed a superb tolerated dose of over 100 mg DOX equiv./kg by the injection of DOX-loaded nanogels with an extraordinary breast tumor accumulation of 8.6%ID/g in mice, exerting effective tumor growth inhibition in MCF-7 human breast tumor-bearing nude mice. These tumor-targeted multifunctional HA nanogels are derived from natural compounds and easy to prepare, rendering them highly appealing for clinical translation.

Pedrosa et al. prepared reducible HA nanogels through self-assembly of 11-amino-1-undecanethiol functionalized HA (HA-AT) followed by crosslinking with 1,4-bis(3-[2-pyridyldithio] propionamido)-butane (DPDPB) via the thiol-disulfide exchange reaction [76]. These HA nanogels showed efficient loading and reduction-controlled release of curcumin and simvastatin. Wu et al. fabricated bioreducible heparin (HEP) nanogels with a size of 80–200 nm by copolymerization of vinyl-functionalized HEP and cystamine bisacrylamide (CBA) without using any surfactants [77]. DOX-loaded HEP nanogels exhibited a remarkable DOX accumulation of 9.3% ID/g in tumors and tumor growth inhibition (TGI) over 85% in the H22 hepatic tumor model. Zhang et al. designed CXCR4

chemokine targeted reducible dextrin nanogels via self-crosslinking of thiolated dextrin followed by coating with AMD3100, a CXCR4 antagonist [78]. These multifunctional dextrin nanogels exhibited a high anti-metastatic effect by inhibiting CXCR4-mediated invasion of 4T1 and U2OS cells. Recently, Zhu et al. developed reducible and fluorescent HA-iodixanol nanogels (HAI-NGs) from HA-cystamine-tetrazole (HA-Cys-Tet) conjugate and polyiodixanol-methacrylate (SS-PI-MA) by combining nanoprecipitation and photo-click crosslinking reaction [79]. HAI-NGs exhibited significantly enhanced X-ray computed tomography (CT) imaging of MCF-7 breast tumors in nude mice following either intratumoral or intravenous injection as compared to free iodixanol control and targeted chemotherapy of MCF-7 human breast tumors.

Chen et al. prepared reduction-responsive polypeptide nanogels by one-step ring-opening polymerization (ROP) of mono-functional phenylalanine *N*-carboxyanhydride and di-functional cysteine *N*-carboxyanhydride [80]. DOX-loaded polypeptide nanogels exhibited an upregulated intratumoral accumulation and improved antitumor efficacy compared with free DOX in a HepG2 hepatoma model. They also designed positively charged reducible polypeptide nanogels based on poly(L-lysine)–poly(L-phenylalanine-co-L-cystine) (PLL–P(LP-co-LC)) [81]. 10-Hydroxycamptothecin (HCPT)-loaded nanogels revealed fast release of HCPT in bladder cancer (BC) cells, prolonged residence time, improved tissue penetration, enhanced antitumor efficacy and reduced side effects as compared to free HCPT in an orthotopic bladder cancer model. Chen et al. fabricated receptor and microenvironment dual-recognizable polypeptide nanogels by decoration with phenylboronic acid (PBA) and morpholine (MP) [82]. DOX-loaded dual-recognizable nanogels exhibited greater targeting efficiency, significantly improved growth inhibition of primary tumor (relative tumor volume was 2.4% compared with PBS control group) as well as markedly reduced metastasis of metastatic B16F10 melanoma over PBA or MP single-modified nanogels (Figure 2).

Reproduced with permission from American Chemical Society [82].

Reducible nanogels are of particular interest for intracellular protein delivery. Chen et al. developed in-situ forming redox-responsive degradable nanogels from water-soluble poly(ethylene glycol)-*b*-poly(2-(hydroxyethyl) methacrylate-*co*-acryloyl carbonate) (PEG-P(HEMA-*co*-AC)) block copolymers under aqueous conditions in the presence of cystamine [83]. These redox-sensitive nanogels exhibited facile loading and enhanced intracellular delivery of cytochrome C (CC), leading to greatly improved apoptosis of HeLa cells compared to free CC or redox-insensitive controls. Li et al. developed cationic reducible nanogels by photo-induced radical polymerization of methacrylated dextran (dex-MA), trimethyl aminoethyl methacrylate (TMAEMA) and pyridyldisulfide methacrylamide for intracellular delivery of OVA into dendritic cells (DCs) [84]. These disulfide-crosslinked nanogels were rapidly dissociated to efficiently release OVA in the presence of cytosol-levels of GSH, which facilitated the cross-presentation of the MHC class

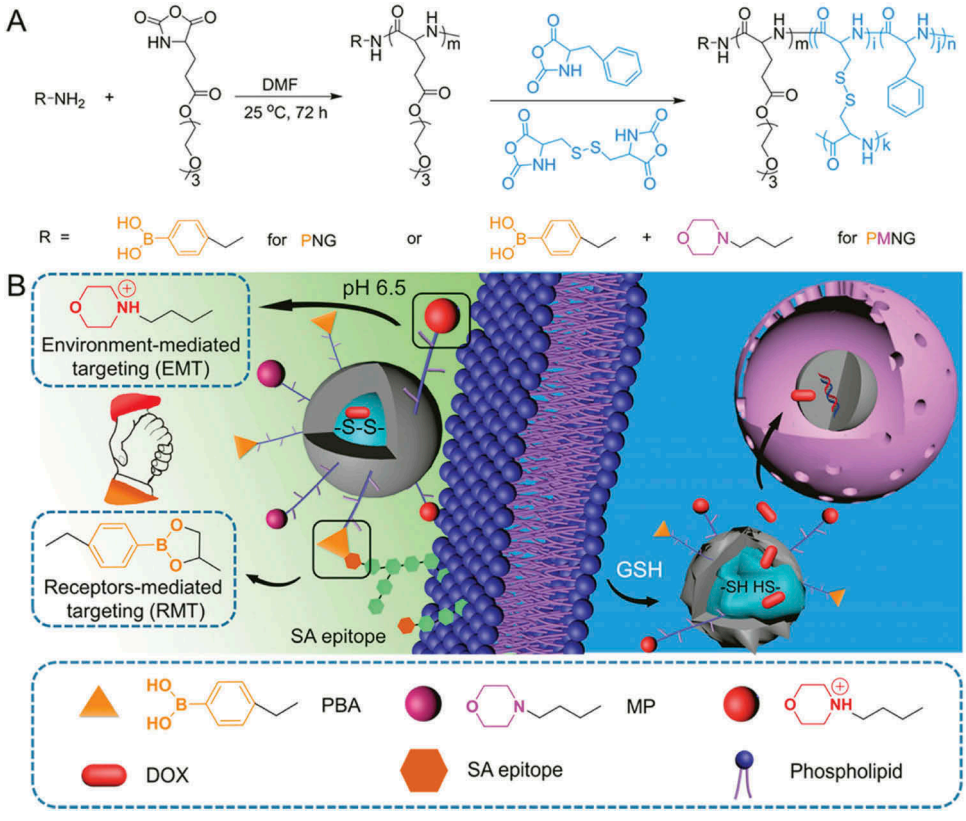


Figure 2. Schematic illustration of engineering nanogels with both receptor-mediated targeting (RMT) and environment-mediated targeting (EMT) for targeted and highly efficient inhibition of metastatic B16F10 melanoma.

Reproduced with permission from American Chemical Society [83].

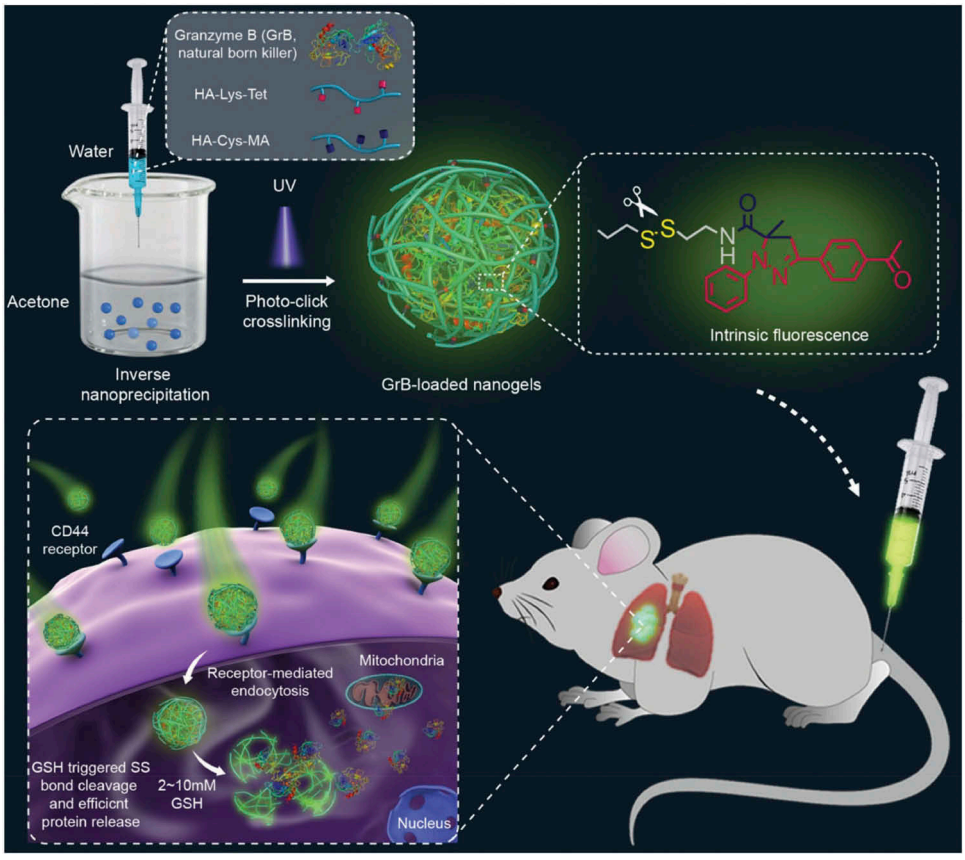


Figure 3. Schematic illustration of bioresponsive fluorescent HA NGs prepared by combining inverse nanoprecipitation and catalyst-free photoclick reaction for facile encapsulation, active tumor-targeting, and intracellular delivery of apoptotic proteins *in vivo*.
Reproduced with permission from American Chemical Society [25].

I antigen in DCs. Chen et al. developed reducible and fluorescent HA nanogels from HA-cystamine-methacrylate (HA-Cys-MA) and HA-lysine-tetrazole (HA-LysTet) by combining inverse nanoprecipitation and photo-click chemistry [25]. These photo-click HA nanogels exhibited a high loading of CC and granzyme B (GrB), and could effectively target and release proteins to CD44 positive MCF-7 and A549 cancer cells (Figure 3). GrB-loaded HA nanogels at a low dose of 3.8–5.7 nmol GrB equiv./kg completely suppressed growth of subcutaneous MCF-7 human breast tumor and orthotopic A549 human lung tumor xenografts with minimal adverse effects. Reducible and fluorescent photo-click HA nanogels were also prepared from hyaluronic acid-graft-oligo(ethylene glycol)-tetrazole (HA-OEG-Tet) using L-cystine dimethacrylamide (MA-Cys-MA) as a crosslinker, which were used to achieve targeted protein therapy of MCF-7 breast tumor in mice with significant tumor growth inhibition at dosages of 80 and 160 nmol CC equiv./kg [85]. More recently, CD44 and EGFR dual-targeted functional nanogels were prepared by incorporating GE11 peptide (YHWYGYTPQNVI)-functionalized HA [26]. These dual-targeted reducible nanogels showed significantly increased uptake by CD44 and EGFR-positive SKOV-3 ovarian cancer cells compared with CD44 single-targeted nanogels. GrB-loaded nanogels induced nearly complete growth suppression of both SKOV-3 human ovarian carcinoma and MDA-MB-231 human breast tumor in mice at a low dose of 3.85 nmol GrB equiv/kg, elucidating that dual targeting approach is potentially interesting in targeted tumor therapy. Reproduced with permission from American Chemical Society [25].

Gouda et al. developed redox-sensitive, PEGylated and cross-linked polyplexes (PCPs) coated with silica nanogels for the intracellular delivery of siRNA [86]. The coating of silica nanogels improved the stability of PCPs, inhibited unintentional siRNA release, and increased the sequence-specific gene silencing activity by promoting endosomal escape and/or regulating the intracellular trafficking. Averick et al. developed reducible cationic nanogels from oligo(ethylene oxide) methacrylate (OEOMA) and quaternized dimethylaminoethyl methacrylate (Q-DMAE) using PEG-dimethacrylate (DMA) as a crosslinker by atom transfer radical polymerization (ATRP) [87]. These cationic nanogels could complex with plasmid DNA and siRNA and mediate reduction-triggered nucleic acid release in the Drosophila Schneider 2 cell line. Hong et al. fabricated reduction-sensitive siRNA/PEI complex nanogels from thiol-terminated siRNA and thiolated linear PEI (LPEI) followed by oxidation [88]. These siRNA/LPEI complex nanogels were highly stable, and exhibited higher cellular uptake by MDA-MB-435-GFP cells and more efficient gene silencing than siRNA/LPEI physical complexes. Dunn et al. designed siRNA conjugated nanogels by photo-crosslinking of siRNA bearing disulfide-linked acrylate groups, and abundant hygroscopic liquid monomers, via a particle replication in non-wetting templates (PRINT)

technique [89]. siRNA was retained well in the cylindrical cationic PRINT nanogels over 48 h in PBS and released fast once arriving at the cytosolic reductive environment, resulting in high transfection efficiency and efficient gene knockdown in HeLa cells. Li et al. designed reduction-sensitive aptamer-based DNA nanogels containing a cancer targeting S6 DNA aptamer for specific recognition of human A549 adenocarcinoma epithelial cell line and release of the therapeutic genes inside the target cells [90]. Nanogels encapsulated with therapeutic genes, antisense oligonucleotides and DNAzyme oligonucleotides, induced marked cell proliferation inhibition, reduction of MMP-9 protein expression, and suppression of A549 cell migration. Nuhn et al. fabricated redox-responsive cationic nanogels from poly(tri(ethylene glycol) methyl ether methacrylate)-b-poly(pentafluorophenyl methacrylate) [P(MEO_3MA)-b-P(PFPMA)] block copolymer using a disulfide-containing spermine derivative as a crosslinker [91]. siRNA was facilely condensed within the cationic nanogels while efficiently released upon addition of DTT due to disruption of the nanogel network. Recently, Li prepared gene concentrated bio-reducible nanogels with locally enriched positive charge but low cytotoxicity for intracellular Bcl2 siRNA delivery [92]. A self-crosslinked reducible siRNA-nanogel complex was formed by reacting thiolated PEI of 1.8 kDa and thiolated dextrin using the suspension method, followed by siRNA loading. The intracellular GSH-triggered siRNA release strategy exhibited the same level of deregulation of Bcl2 protein expression compared with the use of cationic PEI of 25 kDa *in vitro*, while the cytotoxicity was decreased and almost no hemotoxicity was found. Bcl2 siRNA-loaded nanogels significantly inhibited tumor growth in 4T1-luc tumor bearing BALB/C mice, in which the tumor volume was about 24% of the average tumor volume found in the saline group.

6. ROS-responsive nanogels

ROS have been closely related to important pathophysiological events including atherosclerosis, aging, and cancer. A moderate level of ROS is involved with normal cell functions, but excessive amounts of ROS cause oxidative stress and damage of critical components of cells at all levels including DNA, proteins, and lipids. Cancer cells are reported to overproduce ROS and are thus under increased oxidative stress. This phenomenon can be used as one of the promising intracellular stimuli for selective delivery of drugs to diseased sites by targeting oxidative microenvironments at different levels. For example, Shim et al. reported that WIFPWIQL peptide (GRP78P)-functionalized and ROS-responsive thioketal-based DNA complex nanogels caused selective and enhanced gene transfection in prostate cancer cells [93]. Deepagan et al. prepared spontaneous diselenide-crosslinked ROS-responsive nanogels from PEG-polyglutamine derivatives by ROP of γ-3-chloropropanyl-$_L$-glutamate and γ-benzyl-$_L$-glutamate NCA monomers with mPEG-NH2 as a macroinitiator [94]. DOX-loaded ROS-responsive nanogels were shown to release DOX quickly in the presence of 10^{-4} M of H_2O_2 mimicking the tumorous region, resulting in more drug taken up by tumors (3.73-fold higher amount compared with free drug) and effective suppression of tumor growth. Tian et al. developed ROS-responsive nanogel systems by copolymerizing N,N′-bis(methacryloyl) selenocystamine (BMASC) and methacrylic acid (MAA) [95]. These diselenide-crosslinked nanogels were disassembled in response to elevated $^1O_2$ generated by indocyanine green (ICG) with NIR irradiation, resulting in accelerated DOX release in HEK-293 cells and significantly decreased cell viability.

7. Dual-bioresponsive nanogels

Dual-bioresponsive nanogels have received growing interests for targeted drug delivery in that combining two different responses could significantly improve both responsivity and site-specificity [96,97]. Two stimuli take effect either simultaneously at the pathological sites or in a sequential manner in the drug transporting pathways. Dual-responsive nanogels with enhanced control over rate and site of drug release have a tremendous potential for targeted cancer therapy.

7.1. pH and reduction dual-bioresponsive nanogels

pH and redox dual-sensitive nanogels have been designed and developed to achieve (i) fast and complete drug release in cancer cells or (ii) improved cellular uptake via charge reversal at tumor extracellular pH followed by reduction-triggered drug release in the cytosol. pH and reduction dual-responsive polypeptide nanogels were prepared from polyglutamine using 2,2′-dithiobis(N,N′-dimethylethylamine) (dTbDEA) as a crosslinker for intracellular release of DOX in HepG2 cells [98]. The cellular internalization of DOX-loaded nanogels was enhanced under tumor acidic condition (pH 6.8) due to the quaternary ammonium groups. Furthermore, DOX-loaded nanogels exhibited significant *in vitro* and *in vivo* antitumor activities compared with free DOX. Li et al. reported that dual-responsive nanogels prepared from ATRP polymerized PEG-PDEA block polymer crosslinked by N,N′-bis(bromoacetyl) cystamine (BBAC) had enhanced DOX release in an acidic (pH 6.8) and reductive (10.0-mM GSH) environment, leading to more efficient inhibition of A549 cell proliferation [99]. Bahadur et al. constructed pH and redox dual-responsive nanogels from poly[(2-(pyridin-2-yldisulfanyl)-co-PEG] (PDSEG) crosslinked by thiol-disulfide exchange initiated by tris(2-carboxyethyl)phosphine (TCEP) for co-delivery of DOX and PTX to achieve synergetic therapy [100]. The release of DOX from nanogels was accelerated at acidic pH as well as in the reductive environment. The swelling of the nanogels facilitated their escape from the endosomes/lysosomes and DOX delivery into the nucleus, leading to a synergistic effect against HCT-116 cells. Wang et al. developed redox-sensitive nanogels based on dextran grafted PAA with disulfide-containing junctions (Dex-SS-PAA) through a one-step self-assembly assisted methodology (SAA) [101]. DOX was conjugated onto nanogels via an acid-labile hydrazone bond. The release of DOX exhibited pH and redox dual-responsivity, significantly inhibiting the growth of MDA-MB-231 tumors. Wu et al. designed and prepared FA-decorated pH and reduction dual-responsive nanogels from reflux-precipitation copolymerization of AA and CBA and folate PEG conjugation via carbodiimide chemistry as a co-delivery system for DOX and cisplatin (CDDP) to overcome drug resistance [102]. DOX and

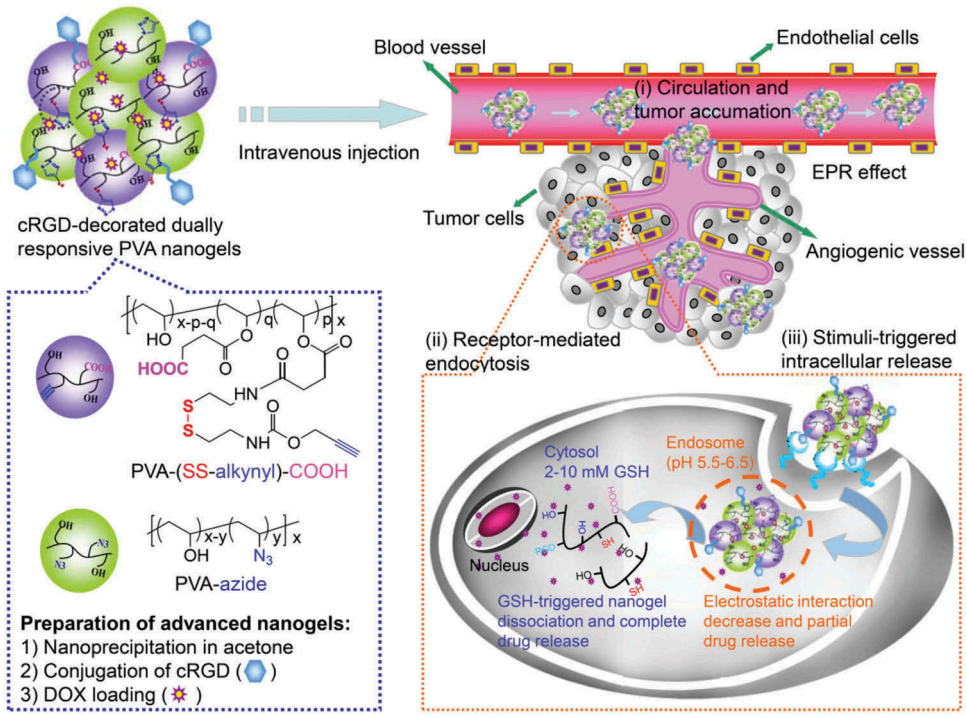


Figure 4. cRGD-decorated pH and reduction dual-responsive PVA nanogels (cRGD-SS-NGs) for efficient treatment of human glioblastoma. Reproduced with permission from Wiley [107].

CDDP-loaded dual-responsive nanogels could introduce more drugs into MCF-7/ADR cells than free drugs, exhibiting a superior cell-killing activity. The *in vivo* test revealed that the combination therapy was effective for the multidrug-resistant MCF-7/ADR tumor with reduced side effects. Thayumanavan et al. prepared self-crosslinked, pH and reduction dual-sensitive nanogels based on PEGMA-*co*-PDPAMA-*co*-PPDSMA copolymer, in which the DPA units exhibited reversible pH-responsive surface charge for enhanced cellular uptake and the disulfide crosslinking points were used for reduction-triggered drug release [103]. The 2-(diisopropylamino)ethyl methacrylate (DPA) moieties in the nanogels became positively charged at tumor pKa of about 6.2, thereby significantly enhancing cellular uptake in HeLa cells.

Haag et al. developed pH and reduction dual-responsive prodrug nanogels from lipoic acid-functionalized hyperbranched polyglycerol (hPG) that was cross-linked with disulfide bonds via a thiol-disulfide exchange reaction and thiol-Michael addition reaction [104]. These nanogels showed low drug leaching while efficient intracellular DOX release via acid-triggered cleavage of the hydrazone linker and dissociation of nanogels triggered by the intracellular GSH was accomplished. pH and reduction dual-responsive PVA nanogels were also obtained from carboxyl-alkynyl-functionalized PVA (PVA-COOH-alkynyl) and azido-functionalized PVA (PVA-N_3) by inverse nanoprecipitation via the click reaction [105]. The introduction of cis-aconitic acid into PVA nanogels not only greatly increased DOX encapsulation due to the strong electrostatic interaction, but also promoted cellular uptake at tumor acidic pH by the surface charge conversion and accelerated DOX release at endosomal pH by the decrease of electrostatic interaction with DOX. The cleavage of disulfide bonds at intracellular GSH concentrations facilitated rapid and complete DOX release. DOX-loaded dual-responsive nanogels had lowIC_{50} values of 0.32 and 0.45 μg DOX equiv./mL for MCF-7 and HeLa cells, respectively. Further studies on cRGD peptide-decorated pH and reduction dual-responsive PVA nanogels revealed efficient and targeted delivery of DOX to $\alpha_v\beta_3$ integrin overexpressing human glioblastoma U87-MG cells *in vitro* and *in vivo*, resulting in effective growth inhibition of a human glioblastoma xenograft in nude mice (Figure 4) [106].

7.2. Other dual-bioresponsive nanogels

pH or redox responsivity has also been combined with other stimuli to obtain dual-bioresponsive nanogels for better controlled drug delivery. For example, ATP, as the 'molecular unit of currency' of intracellular energy transfer, is present in a low concentration (< 0.4 mM) in the extracellular environment, but comparably high concentration in the cytosol (1–10 mM). The prominent difference in ATP levels between the extracellular and intracellular environments can be exploited to trigger drug release from nanogels inside cells [107]. Haag et al. fabricated ATP and pH dual-responsive nanogels from dPG and boronate-functionalized dPG via the inverse nanoprecipitation method [108]. The nanogels were crosslinked via the complexation of 1,2-diols and boronic acids. MTX was efficiently entrapped during the inverse nano-precipitation process. The nanogels could partially release MTX in the acidic endosome/lysosome compartments due to the weakened electrostatic interaction and after arriving at the cytosol, further MTX release took place in response to the cytosolic

ATP concentration. ATP and pH dual-sensitive zwitterionic nanogels were prepared from dPG and citraconic acid-functionalized dPG via inverse nano-precipitation [109]. Notably, these dual-responsive nanogels conferred a two-stage charge conversion profile, i.e. to tumor extracellular conditions (pH 6.5–6.8) for enhanced cellular uptake and to intracellular acidic environment (pH 5–6) for CC release, as well as ATP responsivity to enhance cytosolic release of CC.

Rimondino et al. prepared temperature- and pH-responsive nanogels in a one-pot synthesis from NIPAM, 4-acryloylamine-4-(carboxyethyl)heptanodioic acid (ABC), and *N,N'*-methylene-bisacrylamide (BIS) as a crosslinker for effective encapsulation and sustained release of the anticancer drug cisplatin [110]. Nanogels underwent a reversible volume phase transition triggered by mimicking endolysosomal acidic compartments. Strong hydrogen bonds and a phase change were detected after the deprotonation of ABC moieties, which was exploited for a triggered release of cisplatin at endolysosomal pH values, resulting in high toxicities against A549 cells *in vitro*. Hu et al. designed core-shell nanogels (CS-NGs) based on a core of emulsion polymerized acrylamide (AAm) and *N*-(3-aminopropyl) methacrylamide (APMAAm) with a pH-degradable glycerol dimethacrylate (GDA) crosslinker, and a shell of UV-crosslinked acrylated HA and *N,N'*-methylene-bisacrylamide (MBA) assembled by the carbodiimide crosslinking reaction [111]. These CS-NGs were responsive to overexpressed hyaluronidase (HAase) and acidic pH in the tumor microenvironment for co-delivery of TNF-related apoptosis inducing ligand (TRAIL) and antiangiogenic cilengitide. After intravenous injection into MDA-MB-231 tumor-bearing nude mice, CS-NGs were accumulating in the tumor tissues, where the overexpressed HAase degraded the HA matrix, and previously loaded transglutaminase (TG) was released from the nanogel shells, catalyzing the formation of micro-sized 'drug delivery depots'. The cellular uptake of the oversized depots was restrained, which facilitated the interaction of plasma membranes of the cells with TRAIL/cilengitide released from the depots due to the degradation of nanogel core in the acidic tumor microenvironment.

8. Conclusion

In recent years, we have witnessed rapid evolution of bioresponsive functional nanogels for targeted delivery of anticancer drugs and biotherapeutics. Bioresponsive nanogels are unique in that they have good aqueous stability, can encapsulate different drugs ranging from hydropholic chemotherapeutics to proteins and siRNA, exhibit fast response to biological stimuli, and are amenable to functionalization with cell targeting ligands, hence achieving not only efficient release of drug into the tumor and/or inside the cancer cells but also high cancer cell specificity. Different fabrication methods and crosslinking chemistries allow facile control over nanogel size, drug loading, crosslinking density and drug release rate. The *in vitro* and *in vivo* studies on varying bioresponsive functional nanogels in different cancer cells and tumor models reveal that they are of tremendous potential for improved cancer therapy.

9. Expert opinion

In spite of significant progress achieved in the field of nanogel design and synthesis, few nanogel systems have been translated into clinical studies. There are still many critical issues that need to be investigated for their future clinical applications. In order to be applied in the clinic, bioresponsive nanogels should be made of simple and well-established biocompatible and non-immunogenic materials such as natural polysaccharides, polyesters, polypeptides, PEG, PVA, and small natural compounds (e.g. amino acids, lactic acid, cholesterol, etc.) with as little modification as possible and using biocompatible chemistry. The engineering of nanogels with intricate structures and multi-properties should be performed with a scalable production and a batch-to-batch reproducibility. Microfluidic technologies could be one of the possible methods to address this issue, producing nanogels in a reproducible, well-controlled, and high-throughput manner. It should be noted that bioresponsive nanogel formulations could act like a double-edge sword. If delivered to healthy tissue and cells, nanogels might cause more detrimental effects than traditional non-responsive counterparts. For example, low pH is not only present in the endo/lysosomal compartments of cancer cells, but also in healthy cells. Hence, it is critically important that bioresponsive nanogels are selectively delivered to target tissue and into the tumor cells while sparing normal cells. Tumor-targeted and bioresponsive multifunctional nanogels can be either constructed with an intrinsic targeting ability e.g. by using hyaluronic acid or functionalized with targeting ligands e.g. folic acid, peptides, and antibodies. In this sense, CD44-targeted bioreducible HA nanogels purely based on biocompatible natural raw materials (HA, lipoic acid and amino acids) fabricated via a simple, safe and efficient process demonstrated promising pre-clinical results in different tumor models and are particularly interesting for clinical translation.

One great feature of bioresponsive nanogels is their high water content and excellent compatibility with macromolecular drugs like proteins, siRNA, miRNA, and DNA, which renders them particularly suitable for loading and targeted intracellular delivery of biotherapeutics. The rapid advance in biotechnology has recently identified various proteins, siRNA, miRNA and DNA as new, potent and specific anticancer drugs. The lack of safe and efficient delivery systems, however, restrains their advance to clinical settings. In the future, more efforts should be directed to the development of clinically viable bioresponsive functional nanogels for targeted delivery and triggered cytosolic release of therapeutic proteins, siRNA, miRNA and DNA. Moreover, bioresponsive functional nanogels are also of particular interest for co-delivery of small chemical anticancer agents and macromolecular drugs, to effectively combat drug resistance and metastasis that are responsible for most incurable cancers. At the moment most nanogels have been developed only containing one ligand for targeting to tumors. In the future more attention has to paid to the design of nanogels with multiple ligands to improve targeting, transport through blood vessels via transcytosis to the tumor, tumor penetration and uptake by tumor cells.

It should also be noted that to date little is known about the exact extracellular and intracellular fate of the bio-responsive nanogels, especially with respect to the extracellular

enzyme-triggered degradation, the behavior within the endo/lysosomal compartments and the lack of detailed understanding of disulfide cleavage in the cytosol. Further development of responsive nanogel systems requires better understanding of their extracellular/intracellular trafficking and fate. Moreover, although many studies proved the efficacy of nanogel formulations and their safety, detailed information about their long-term accumulation, *in vivo* pharmacokinetics and degradation profiles is still lacking. Hopefully, future studies also using more advanced analytical techniques will provide us with better insights about the *in vitro* and *in vivo* behavior of nanogels and will help to translate the improvements in nanogel design and development of nanogel formulations from the bench to the bedside. We are convinced that with rational design and further intensive studies, bioresponsive polymeric nanogel formulations will evolve as highly promising tools for the effective treatment of various cancers in clinical trials.

Funding

This work was supported by the National Key Research and Development Program of China [grant number 2017YFD0401301], [grant number 2017YFD0400203], [grant number 2017YFD0400402]; the National Natural Science Foundation of China (NSFC) [grant number 51703244], [grant number 81600178]; the Natural Science Foundation of Jiangsu Province [grant number BK20170730]; the Fundamental Research Funds for the Central Universities [grant number 2632017ZD01]; and the Jiangsu Specially-Appointed Professors Program to W Chen.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer declaration of interest

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Kabanov AV, Vinogradov SV. Nanogels as pharmaceutical carriers: finite networks of infinite capabilities, Angew. Chem Int Ed. 2009;48:5418–5429.
2. Eckmann DM, Composto RJ, Tsourkas A, et al. Nanogel carrier design for targeted drug delivery. J Mater Chem B. 2014;2:8085–8097.
3. Li D, Van Nostrum CF, Mastrobattista E, et al. Nanogels for intracellular delivery of biotherapeutics. J Control. Release. 2017;259:16–28.
4. Chacko RT, Ventura J, Zhuang J, et al. Polymer nanogels: A versatile nanoscopic drug delivery platform. Adv Drug Deliv Rev. 2012;64:836–851.
5. Raemdonck K, Demeester J, De Smedt S. Advanced nanogel engineering for drug delivery. Soft Matter. 2009;5:707–715.
6. Zhang H, Zhai Y, Wang J, et al. New progress and prospects: the application of nanogel in drug delivery. Sci Eng C Mater Biol Appl. 2016;60:560–568.
7. Deng C, Jiang Y, Cheng R, et al. Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: promises, progress and prospects. Nano Today. 2012;7:467–480.
8. Sun T, Zhang YS, Pang B, et al. Engineered nanoparticles for drug delivery in cancer therapy. Angew Chem Int Ed. 2014;53:12320–12364.
9. Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. Nat Rev Drug Discov. 2014;13:813.
10. Cheng R, Meng F, Deng C, et al. Bioresponsive polymeric nanotherapeutics for targeted cancer chemotherapy. Nano Today. 2015;10:656–670.
11. Lu Y, Aimetti AA, Langer R, et al. Bioresponsive materials. Nat Rev Mater. 2017;2:16075.
12. Zha L, Banik B, Alexis F. Stimulus responsive nanogels for drug delivery. Soft Matter. 2011;7:5908–5916.
13. Smith MH, Lyon LA. Multifunctional nanogels for siRNA delivery. Acc Chem Res. 2012;45:985–993.
14. Jhaveri A, Deshpande P, Torchilin V. Stimuli-sensitive nanopreparations for combination cancer therapy. J Control. Release. 2014;190:352–370.
15. Li Y, Maciel D, Rodrigues J, et al. Biodegradable polymer nanogels for drug/nucleic acid delivery. Chem Rev. 2015;115:8564–8608.
16. Oh JK, Drumright R, Siegwart DJ, et al. The development of microgels/nanogels for drug delivery applications. Prog Polym Sci. 2008;33:448–477.
17. Oh JK, Lee DI, Park JM. Biopolymer-based microgels/nanogels for drug delivery application. Prog Polym Sci. 2009;34:1261–1282.
18. Liu D, Zhang H, Fontana F. et al. Microfluidic-assisted fabrication of carriers for controlled drug delivery. Lab on a Chip. 2017;17:1856–1883.
19. Bazban-Shotorbani S, Dashtimoghadam E, Karkhaneh A, et al. Microfluidic directed synthesis of alginate nanogels with tunable pore size for efficient protein delivery. Langmuir. 2016;32:4996–5003.
20. Ko DY, Shinde UP, Yeon B, et al. Recent progress of in situ formed gels for biomedical applications. Prog Polym Sci. 2013;38:672–701.
21. Zhang X, Malhotra S, Molina M, et al. Micro- and nanogels with labile crosslinks-from synthesis to biomedical applications. Chem Soc Rev. 2015;44:1948–1973.
22. Jiang Y, Chen J, Deng C, et al. Click hydrogels, microgels and nanogels: emerging platforms for drug delivery and tissue engineering. Biomaterials. 2014;35:4969–4985.
23. Steinhilber D, Rossow T, Wedepohl S, et al. A microgel construction kit for bioorthogonal encapsulation and pH-controlled release of living cells. Angew Chem Int Ed. 2013;52:13538–13543.
24. Steinhilber D, Witting M, Zhang X, et al. Surfactant free preparation of biodegradable dendritic polyglycerol nanogels by inverse nanoprecipitation for encapsulation and release of pharmaceutical biomacromolecules, J. Control. Release. 2013;169:289–295.
25. Chen J, Zou Y, Deng C, et al. Multifunctional click hyaluronic acid nanogels for targeted protein delivery and effective cancer treatment in vivo. Chem Mater. 2016;28:8792–8799.
•• **Paper reporting advanced reducible nanogels with intrinsic fluorescence for trageted protein therapy and cell imaging.**
26. Chen J, Ouyang J, Chen Q, et al. EGFR and CD44 dual-targeted multifunctional hyaluronic acid nanogels boost protein delivery to ovarian and breast cancers in vitro and in vivo. ACS Appl Mater Interface. 2017;9:24140–24147.
27. Soni KS, Desale SS, Bronich TK. Nanogels: an overview of properties, biomedical applications and obstacles to clinical translation. J Control. Release. 2016;240:109–126.
28. Neamtu I, Rusu AG, Diaconu A, et al. Basic concepts and recent advances in nanogels as carriers for medical applications. Drug Deliv. 2017;24:539–557.
29. Sivaram AJ, Rajitha P, Maya S, et al. Nanogels for delivery, imaging and therapy, Wiley Interdiscip. Rev Nanomed Nanobiotechnol. 2015;7:509–533.
30. Wu H-Q, Wang -C-C. Biodegradable smart nanogels: A new platform for targeting drug delivery and biomedical diagnostics. Langmuir. 2016;32:6211–6225.
31. Ding L, Jiang Y, Zhang J, et al. pH-sensitive coiled-coil peptide-cross-linked hyaluronic acid nanogels: synthesis and targeted intracellular protein delivery to CD44 positive cancer cells. Biomacromolecules. 2018;19:555–562.
32. Binauld S, Stenzel MH. Acid-degradable polymers for drug delivery: A decade of innovation. Chem Commun. 2013;49:2082–2102.

33. Zhu X, Sun Y, Chen D, et al. Mastocarcinoma therapy synergistically promoted by lysosome dependent apoptosis specifically evoked by 5-Fu@nanogel system with passive targeting and pH activatable dual function. J Control. Release. 2017;254:107–118.
34. Arunraj TR, Sanoj Rejinold N, Ashwin Kumar N, et al. Doxorubicin-chitin-poly(caprolactone) composite nanogel for drug delivery. Inter J Biolog Macromol. 2013;62:35–43.
35. Khaled SZ, Cevenini A, Yazdi IK, et al. One-pot synthesis of pH-responsive hybrid nanogel particles for the intracellular delivery of small interfering RNA. Biomaterials. 2016;87:57–68.
36. Lee ES, Kim D, Youn YS, et al. A virus-mimetic nanogel vehicle. Angew Chem. 2008;120:2452–2455.
37. Ju C, Mo R, Xue J, et al. Sequential intra-intercellular nanoparticle delivery system for deep tumor penetration. Angew Chem Int Ed. 2014;53:6253–6258.
38. Song Q, Yin Y, Shang L, et al. Tumor microenvironment responsive nanogel for the combinatorial antitumor effect of chemotherapy and immunotherapy. Nano Lett. 2017;17:6366–6375.
• **Paper reporting pH-degradable nanogel formulations for efficient combination chemotherapy and immunotherapy.**
39. Mavuso S, Marimuthu T, Choonara YE, et al. A review of polymeric colloidal nanogels in transdermal drug delivery. Curr Pharm Des. 2015;21:2801–2813.
40. Mangalathillam S, Rejinold NS, Nair A, et al. Curcumin loaded chitin nanogels for skin cancer treatment via the transdermal route. Nanoscale. 2012;4:239–250.
41. Divya G, Panonnummal R, Gupta S, et al. Acitretin and aloe-emodin loaded chitin nanogel for the treatment of psoriasis. Eur J Pharm Biopharm. 2016;107:97–109.
42. Gao C, Lin Z, Jurado-Sánchez B, et al. Stem cell membrane-coated nanogels for highly efficient in vivo tumor targeted drug delivery. Small. 2016;12:4056–4062.
43. Yahia-Ammar A, Sierra D, Mérola F, et al. Self-assembled gold nanoclusters for bright fluorescence imaging and enhanced drug delivery. ACS Nano. 2016;10:2591–2599.
44. Li X, Du P, Liu P. Layer-by-layer polyelectrolyte complex coated poly(methacrylic acid) nanogels as a drug delivery system for controlled release: structural effects. RSC Adv. 2014;4:56323–56331.
45. Lin L, Xu W, Liang H, et al. Construction of pH-sensitive lysozyme/pectin nanogel for tumor methotrexate delivery. Colloids Surf B Biointerfaces. 2015;126:459–466.
46. Su S, Wang H, Liu X, et al. iRGD-coupled responsive fluorescent nanogel for targeted drug delivery. Biomaterials. 2013;34:3523–3533.
47. Wu W, Aiello M, Zhou T, et al. In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. Biomaterials. 2010;31:3023–3031.
48. Wu W, Shen J, Banerjee P, et al. Chitosan-based responsive hybrid nanogels for integration of optical pH-sensing, tumor cell imaging and controlled drug delivery. Biomaterials. 2010;31:8371–8381.
49. Du J-Z, Sun T-M, Song W-J, et al. A tumor-acidity-activated charge-conversional nanogel as an intelligent vehicle for promoted tumoral-cell uptake and drug delivery. Angew Chem. 2010;122:3703–3708.
50. Chen W, Hou Y, Tu Z, et al. pH-degradable PVA-based nanogels via photo-crosslinking of thermo-preinduced nanoaggregates for controlled drug delivery, J. Control. Release. 2017;259:160–167.
• **Paper reporting pH-degradable nanogels for triggered drug release with the native degradation product.**
51. Li Y, Bui QN, Duy LTM. et al. One-step preparation of pH-responsive polymeric nanogels as intelligent drug delivery systems for tumor therapy, Biomacromolecules. 2018;19:2062–2070.
52. Dimde M, Neumann F, Reisbeck F, et al. Defined pH-sensitive nanogels as gene delivery platform for siRNA mediated in vitro gene silencing. Biomater Sci. 2017;5:2328–2336.
53. Ulijn RV. Enzyme-responsive materials: A new class of smart biomaterials. J Mater Chem. 2006;16:2217–2225.
54. De La Rica R, Aili D, Stevens MM. Enzyme-responsive nanoparticles for drug release and diagnostics. Adv Drug Deliv Rev. 2012;64:967–978.
55. Hu J, Zhang G, Liu S. Enzyme-responsive polymeric assemblies, nanoparticles and hydrogels. Chem Soc Rev. 2012;41:5933–5949.
56. Yang C, Wang X, Yao X, et al. Hyaluronic acid nanogels with enzyme-sensitive cross-linking group for drug delivery. J Control. Release. 2015;205:206–217.
57. Jiang T, Mo R, Bellotti A, et al. Gel-liposome-mediated co-delivery of anticancer membrane-associated proteins and small-molecule drugs for enhanced therapeutic efficacy. Adv Funct Mater. 2014;24:2295–2304.
•• **Paper reporting programmed drug-delivery nanogel-liposome system for sequential and site-specific drug delivery and efficeint cancer therapy.**
58. Qiuwen Z, Xiaojie C, Xiao X, et al. Tumor-specific self-degradable nanogels as potential carriers for systemic delivery of anticancer proteins. Adv Funct Mater. 2018;28:1707371.
59. Wang Y, Luo Y, Zhao Q, et al. An enzyme-responsive nanogel carrier based on PAMAM dendrimers for drug delivery. ACS Appl Mater Interface. 2016;8:19899–19906.
60. Xiong M-H, Bao Y, Du X-J, et al. Differential anticancer drug delivery with a nanogel sensitive to bacteria-accumulated tumor artificial environment. ACS Nano. 2013;7:10636–10645.
61. Wang Y, Qiao S-L, Wang H. Facile synthesis of peptide cross-linked nanogels for tumor metastasis inhibition. ACS Appl Nano Mater. 2018;1:785–792.
62. Wang Y, Xu H, Wang J, et al. Development of a thermally responsive nanogel based on chitosan-poly(N-isopropylacrylamide-co-acrylamide) for paclitaxel delivery. J Pharm Sci. 2014;103:2012–2021.
63. Molina M, Wedepohl S, Miceli E, et al. Overcoming drug resistance with on-demand charged thermoresponsive dendritic nanogels. Nanomedicine. 2017;12:117–129.
64. Shirakura T, Kelson TJ, Ray A, et al. Hydrogel nanoparticles with thermally controlled drug release. ACS Macro Lett. 2014;3:602–606.
65. Meng F, Hennink WE, Zhong Z. Reduction-sensitive polymers and bioconjugates for biomedical applications. Biomaterials. 2009;30:2180–2198.
66. Cheng R, Feng F, Meng F, et al. Glutathione-responsive nanovehicles as a promising platform for targeted intracellular drug and gene delivery. J Control Release. 2011;152:2–12.
67. Arunachalam B, Phan UT, Geuze HJ, et al. Enzymatic reduction of disulfide bonds in lysosomes: characterization of a Gamma-interferon-inducible lysosomal thiol reductase (GILT). Proc Natl Acad Sci USA. 2000;97:745–750.
68. Li Y-L, Zhu L, Liu Z, et al. Reversibly stabilized multifunctional dextran nanoparticles efficiently deliver doxorubicin into the nuclei of cancer cells. Angew Chem Int Ed. 2009;48:9914–9918.
69. Chen W, Zhong P, Meng F, et al. Redox and pH-responsive degradable micelles for dually activated intracellular anticancer drug release. J Control Release. 2013;169:171–179.
70. Chen W, Zou Y, Jia J, et al. Functional poly(ε-caprolactone)s via copolymerization of ε-caprolactone and pyridyl disulfide-containing cyclic carbonate: controlled synthesis and facile access to reduction-sensitive biodegradable graft copolymer micelles. Macromolecules. 2013;46:699–707.
71. Ryu J-H, Jiwpanich S, Chacko R, et al. Surface-functionalizable polymer nanogels with facile hydrophobic guest encapsulation capabilities . J Am Chem Soc. 2010;132:8246–8247.
72. ParK H, Choi Y, Jeena M, et al. Reduction-triggered self-cross-linked hyperbranched polyglycerol nanogels for intracellular delivery of drugs and proteins. Macromol Biosci. 2018;18:1700356.
73. Zhong Y, Zhang J, Cheng R, et al. Reversibly crosslinked hyaluronic acid nanoparticles for active targeting and intelligent delivery of doxorubicin to drug resistant CD44+ human breast tumor xenograft. J Control Release. 2015;205:144–154.
•• **Paper reporting reducible hyaluronic acid nanogels totally composed of natural materials for active targeting and effective therapy on CD44-positive breast cancers.**
74. Zhong Y, Meng F, Deng C, et al. Targeted inhibition of human hematological cancers in vivo by doxorubicin encapsulated in smart lipoic acid-crosslinked hyaluronic acid nanoparticles. Drug Deliv. 2017;24:1482–1490.

75. Sun B, Deng C, Meng F. et al. Robust, active tumor-targeting and fast bioresponsive anticancer nanotherapeutics based on natural endogenous materials. Acta Biomater. 2016;45:223–233.
76. Pedrosa SS, Gonçalves C, David L, et al. A novel crosslinked hyaluronic acid nanogel for drug delivery. Macromol Biosci. 2014;14:1556–1568.
77. Wu W, Yao W, Wang X, et al. Bioreducible heparin-based nanogel drug delivery system. Biomaterials. 2015;39:260–268.
78. Zhang F, Gong S, Wu J, et al. CXCR4-targeted and redox responsive dextrin nanogel for metastatic breast cancer therapy. Biomacromolecules. 2017;18:1793–1802.
79. Zhu Y, Wang X, Chen J, et al. Bioresponsive and fluorescent hyaluronic acid-iodixanol nanogels for targeted X-ray computed tomography imaging and chemotherapy of breast tumors. J Control. Release. 2016;244:229–239.
80. Huang K, Shi B, Xu W. et al. Reduction-responsive polypeptide nanogel delivers antitumor drug for improved efficacy and safety. Acta Biomater. 2015;27:179–193.
81. Guo H, Xu W, Chen J, et al. Positively charged polypeptide nanogel enhances mucoadhesion and penetrability of 10-hydroxycamptothecin in orthotopic bladder carcinom. J Control. Release. 2017;259:136–148.
82. Chen J, Ding J, Xu W, et al.. Receptor and microenvironment dual-recognizable nanogel for targeted chemotherapy of highly metastatic malignancy. Nano Lett. 2017;17:4526–4533.
83. Chen W, Zheng M, Meng F, et al. In situ forming reduction-sensitive degradable nanogels for facile loading and triggered intracellular release of proteins. Biomacromolecules. 2013;14:1214–1222.
84. Li D, Kordalivand N, Fransen MF, et al. Reduction-sensitive dextran nanogels aimed for intracellular delivery of antigens. Adv Funct Mater. 2015;25:2993–3003.
85. Li S, Zhang J, Deng C, et al. Redox-sensitive and intrinsically fluorescent photoclick hyaluronic acid nanogels for traceable and targeted delivery of cytochrome c to breast tumor in mice. ACS Appl Mater Interface. 2016;8:21155–21162.
86. Gouda N, Miyata K, Christie RJ, et al. Silica nanogelling of environment-responsive PEGylated polyplexes for enhanced stability and intracellular delivery of siRNA. Biomaterials. 2013;34:562–570.
87. Averick SE, Paredes E, Irastorza A, et al. Preparation of cationic nanogels for nucleic acid delivery. Biomacromolecules. 2012;13:3445–3449.
88. Hong CA, Kim JS, Lee SH, et al. Reductively dissociable siRNA-polymer hybrid nanogels for efficient targeted gene silencing. Adv Funct Mater. 2013;23:316–322.
89. Dunn SS, Tian S, Blake S, et al. Reductively responsive siRNA-conjugated hydrogel nanoparticles for gene silencing. J Am Chem Soc. 2012;134:7423–7430.
90. Li J, Zheng C, Cansiz S, et al. Self-assembly of DNA nanohydrogels with controllable size and stimuli-responsive property for targeted gene regulation therapy. J Am Chem Soc. 2015;137:1412–1415.
91. Nuhn L, Braun L, Overhoff I, et al. Degradable cationic nanohydrogel particles for stimuli-responsive release of siRNA. Macromol Rapid Commun. 2014;35:2057–2064.
92. Li H, Yang X, Gao F. et al. Bioreduction-ruptured nanogel for switch on/off release of Bcl2 siRNA in breast tumor therapy. J ControlRelease. 2018. doi:10.1016/j.jconrel.2018.02.036
93. Shim MS, Xia Y. A reactive oxygen species (ROS)-responsive polymer for safe, efficient, and targeted gene delivery in cancer cells. Angew Chem Int Ed. 2013;52:6926–6929.
94. Deepagan VG, Kwon S, You DG, et al. In situ diselenide-crosslinked polymeric micelles for ROS-mediated anticancer drug delivery. Biomaterials. 2016;103:56–66.
95. Tian Y, Zheng J, Tang X, et al. Near-Infrared Light-Responsive Nanogels with Diselenide-Cross-Linkers for On-Demand Degradation and Triggered Drug Release. Part Syst Char. 2015;32:547–551.
96. Cheng R, Meng F, Deng C, et al. Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery. Biomaterials. 2013;34:3647–3657.
97. Chen W, Meng F, Cheng R, et al. Facile construction of dual-bioresponsive biodegradable micelles with superior extracellular stability and activated intracellular drug release. J Control. Release. 2015;210:125–133.
98. Ding J, Xu W, Zhang Y, et al. Self-reinforced endocytoses of smart polypeptide nanogels for "on-demand" drug delivery. J Control. Release. 2013;172:444–455.
99. Li M, Tang Z, Sun H, et al. pH and reduction dual-responsive nanogel cross-linked by quaternization reaction for enhanced cellular internalization and intracellular drug delivery. Polym Chem. 2013;4:1199–1207.
100. Bahadur K. C R, Xu P, Multicompartment intracellular self-expanding nanogel for targeted delivery of drug cocktail. Adv Mater. 2012;24:6479–6483.
101. Wang H, Dai T, Zhou S, et al. Self-assembly assisted fabrication of dextran-based nanohydrogels with reduction-cleavable junctions for applications as efficient drug delivery systems. Sci Rep. 2017;7:40011.
102. Wu H, Jin H, Wang C, et al. Synergistic cisplatin/doxorubicin combination chemotherapy for multidrug-resistant cancer via polymeric nanogels targeting delivery. ACS Appl Mater Interface. 2017;9:9426–9436.
103. Li L, Raghupathi K, Yuan C, et al. Surface charge generation in nanogels for activated cellular uptake at tumor-relevant pH. Chem Sci. 2013;4:3654–3660.
104. Zhang X, Achazi K, Steinhilber D, et al. A facile approach for dual-responsive prodrug nanogels based on dendritic polyglycerols with minimal leaching. J Control. Release. 2014;174:209–216.
105. Chen W, Achazi K, Schade B, et al. Charge-conversional and reduction-sensitive poly(vinyl alcohol) nanogels for enhanced cell uptake and efficient intracellular doxorubicin release . J Control. Release. 2015;205:15–24.
106. Chen W, Zou Y, Zhong Z, et al. Cyclo(RGD)-decorated reduction-responsive nanogels mediate targeted chemotherapy of integrin overexpressing human glioblastoma in vivo. Small. 2017;13:1601997–1602005.
•• **Paper reporting ligand-decorated dual-responsive nanogels for targeted drug delivery and efficient cancer therapy.**
107. Mo R, Jiang T, DiSanto R, et al. ATP-triggered anticancer drug delivery. Nat Commun. 2014;5:3364.
108. Zhang X, Achazi K, Haag R. Boronate cross-linked ATP- and pH-responsive nanogels for intracellular delivery of anticancer drugs. Adv Healthc. 2015;4:585–592.
109. Zhang X, Zhang K, Haag R. Multi-stage, charge conversional, stimuli-responsive nanogels for therapeutic protein delivery. Biomater Sci. 2015;3:1487–1496.
110. Rimondino GN, Miceli E, Molina M, et al. Rational design of dendritic thermoresponsive nanogels that undergo phase transition under endolysosomal conditions. J Mater Chem B. 2017;5:866–874.
111. Hu Q, Sun W, Lu Y. et al. Tumor microenvironment-mediated construction and deconstruction of extracellular drug-delivery depots. Nano Lett. 2016;16(1118–1126).