Nanogels: an overview of properties, biomedical applications and obstacles to clinical translation

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Graphical abstract
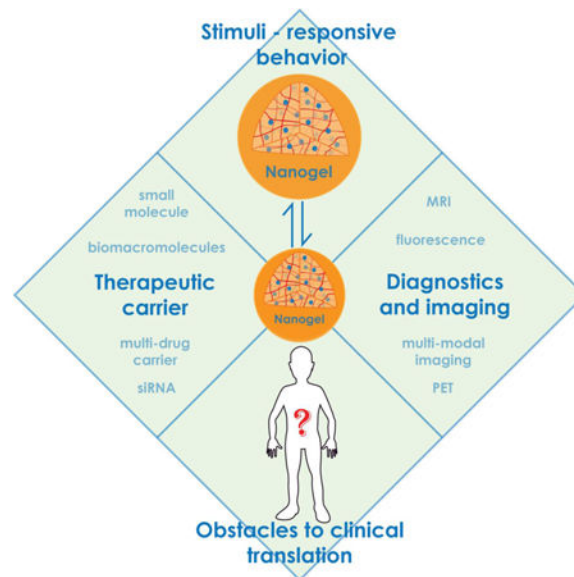
1. Introduction

Nanogels are three-dimensional hydrogel materials in the nanoscale size range formed by crosslinked swellable polymer networks with a high capacity to hold water, without actually dissolving into the aqueous medium. Nanogels can be composed of a variety of naturally occurring polymers, synthetic polymers or a combination thereof. Their characteristics such as size, charge, porosity, amphiphilicity, softness, and degradability can be fine-tuned by varying the chemical composition of the nanogels. They are mostly spherical particles but the current advancement in synthetic strategies allow for the fabrication of nanogels of different shapes [1,2]. They can be also designed to have either a core-shell or a core-shell-

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corona structure, with at least one of the layers crosslinked for structural integrity. Being mostly hydrophilic in nature, nanogels are highly biocompatible with a high loading capacity for guest molecules and their unique physical properties offer them distinct advantages over other types of nanomaterials for biomedical applications. Nanogels not only protect the cargo from degradation and elimination but also participate actively in the delivery process due to their characteristic properties like stimuli-responsive behavior, softness and swelling to help achieve a controlled, triggered response at the target site [3-9]. The versatility of their architecture allows for incorporation of a plethora of guest molecules ranging from inorganic nanoparticles to biomacromolecules like proteins and DNA with suitable modifications of the materials used for their construction, without compromising their gel-like behavior [10-16]. This multifunctionality and stability is hard to find in other types of nanoparticulate systems [17,18]; especially the ability to incorporate entities with very different physical properties within the same carrier. Inorganic nanomaterials have distinct material properties like optical activity, electrical conductivity and magnetic properties that make them suitable for *in vivo* diagnostic and imaging applications, but they suffer from limitations of poor colloidal stability, low aqueous solubility and rapid elimination by the mononuclear phagocytic system (MPS). Polymeric nanogels can be used as carriers for such imaging probes by imparting stability and increasing their utility. This lead to the evolution of a new class of agents termed 'nanohybrids' which are nanogels incorporating inorganic materials [19,20]. Such nanohybrids can contain a wide variety of diagnostic and imaging agents for different types of medical conditions. Nanogels prevent biomolecules like enzymes and genetic material from degradation while their own macromolecular properties help increase the circulation half-lives of small molecules, and serve as a highly convenient platform for combination delivery of therapeutic molecules. They can be targeted specifically to the site of interest by conjugation with a targeting ligand or due to the passive targeting that is a characteristic feature of their nanoscale size. Despite such diversity in their applications, nanogels are not yet a part of clinical use. Many comprehensive and more specialized review articles on synthesis and application of nanogels were recently published. For that reason, in the present paper we attempted to briefly consider characteristic features of nanogels and to demonstrate representative examples for major directions in their applications in the biomedical field. We also highlight some of the key hurdles that need to be overcome to make nanogels a part of routine clinical practice.

2. Nanogels: characteristic features

2.1 Synthesis of nanogels

Nanogels can be synthesized by a number of techniques. Since an in-depth discussion of all the available techniques is beyond the scope of this review, a brief overview of the techniques is given, along with reference to more detailed sources. Traditionally, nanogels have been classified based on the method of crosslinking as either physically or chemically (covalently) crosslinked nanogels. Chemical crosslinking involves formation of covalent bonds between the polymer chains during polymerization of low molecular weight monomers or crosslinking of polymer precursors. The most extensively employed methods for preparing chemically crosslinked nanogels utilize heterogeneous polymerization

reactions in the presence of either bifunctional or multifunctional crosslinkers [21,22]. Conventional and controlled/living radical polymerization techniques allow for preparation of nanogels with different composition, dimensions, and architectures including core-shell and hollow nanogel particles [23,24]. The use of functional initiators and macroinitiators further allows the incorporation of functionalities in the interior or on the surface of nanogels, which facilitate multivalent bioconjugation [25]. A variety of other crosslinking approaches including click chemistry, Schiff-base reactions, thiol-disulfide exchange, amide crosslinking, photo-induced crosslinking, enzyme-mediated crosslinking etc., have been developed for the synthesis of nanogels from the polymer precursors. In addition, the crosslinking reactions carried out on preformed core-shell self-assemblies such as polymer micelles allow introducing a high degree of spatial organization into the nanogels [26,27]. For a comprehensive look into crosslinking strategies, the reader is directed to excellent review written by Haag and colleagues [28]. Recent advances in nanoscale fabrication methods provided further unique opportunities to fabricate well-defined nanogels with precise control over size, shape, deformability and surface chemistry in a high-throughput manner [29-31]. Physically crosslinked systems though formed under the mild conditions, tend to be more fragile than their covalently crosslinked counterparts since they are stabilized by relatively weak interactions between polymer chains such as hydrogen bonding, hydrophobic interactions or ionic interactions. The use of hydrophobically-modified polymers and other associating polymers for preparing functional nanogels has recently been reviewed by Sasaki and Akiyoshi [32]. One of the challenges in the formation of nanogels by such polymers is a control over the particle size, which requires fine-tuning of the polymer concentrations or environmental parameters such as temperature, pH, and ionic strength. A study by Nielsen and co-workers has demonstrated that these challenges can be addressed by utilizing a microfluidics-based approach [33]. Overall, the advances in polymer chemistry led to the exceptional diversity and control over the composition, architecture and functionality of crosslinked nanogels, which in turn provide more flexibility to tune their properties to comply with targeted biomedical applications.

2.2 Stimuli-responsive behavior

Stimuli-responsive behavior of nanogels is a sequence of events initiated by an external cue that comes either from the specific environment within the body like change in pH, temperature, redox conditions or enzyme concentration, or a stimulus that can be applied externally such as light, magnetic field, etc. This stimulus then causes a conformational or structural change in the nanogel, which is mediated by various factors including, but not limited to, transition in the temperature below or above its lower critical solution temperature (LCST) or the ionization of acidic or basic functional groups on the polymer chains. These changes also alter the hydrophilicity and/or hydrophobicity of the nanogels or, in other words, the extent of interaction of the system with water molecules [34-36] and is mostly manifested in the form of swelling or deswelling of the nanogel network, which in turn causes responses like release of the entrapped cargo [37,38]. One of the considerable advantages of nanogels over the macroscopic gels is their very rapid response to a change in environmental conditions [39]. The responsiveness of nanogels to the external physical or chemical signals can be tightly regulated by controlling the structure of the materials used for preparation of the nanogels [3,7]. For example, in the case of polyelectrolyte nanogels

pH-dependent ionization of functional groups results in an increase of osmotic pressure inside the nanogel due to entrapped counterions and ultimately results in the swelling of the nanogels [7,40,41]. It is well recognized that a balance between the osmotic pressure and the polymer elasticity sets the physical dimensions of a hydrogel particle [42]. Thus, the extent of swelling also depends on structural characteristics of the nanogel like the chemical composition, hydrophilicity of crosslinkers, and the degree of crosslinking of the nanogel network, which controls the freedom of conformational mobility of the polymer chains [43,44]. Tan et al. studied pH-responsive polyampholyte nanogels with different fractions of methacrylic acid (MAA) and 2-diethylaminoethyl methacrylate (DEAM) units in the crosslinked polymer cores that were sterically stabilized by poly(ethylene glycol), PEG, shell [45]. The MAA segment in this nanogel is a weak acid and DEAM is a weak base (pKa = 5.4 for MAA and pKa = 7.3 for DEAM). The DEAM segments could be protonated at low pH, imparting a positive charge to this segment, whereas the MAA units are negatively charged at high pH. As a result, these nanogels exhibited marked swelling at both high and low pH values, but shrunk in the vicinity of isoelectric point due to overall charge neutralization. It was demonstrated that swelling-deswelling transition can be tuned by varying the composition of MAA and DEAM in the nanogels. The unique charge-switching properties of amphoteric nanogels based on poly(N-isopropylacrylamide) functionalized with aminophenylboronic acid (PBA) were explored by Hoare and Pelton to regulate the release of preloaded insulin in glucose-dependent manner [46]. The binding of glucose to the PBA residues shifts the boronic acid ionization equilibrium, increases the anionic charge density on the gel and drives a gel swelling response. These nanogels were designed to both swell and deswell in response to glucose according to the pH of the medium, the concentration of PBA groups grafted to the nanogel, and the relative concentrations of the cationic and anionic functional groups in the platform nanogel. Such PBA-nanogels had a high capacity for insulin uptake and selectively released insulin under physiological conditions in an "on-off" manner with fluctuating glucose level. Similar approach was adapted by Wu et al. for the design of a nanogel-based glucose sensor, which comprised of a core of Ag nanoparticle encapsulated within a glucose-recognizing crosslinked shell containing PBA residues. The swelling of the gel in response to glucose binding causes a change in the refractive index of the medium around Ag nanoparticle that leads to change in its fluorescence properties. The response rate is reported to be on the order of 100 ns, and the detection sensitivity for change in glucose concentration was reported to be ± 0.1 mM [47]. Thus, both sensitivity and rate of response of the nanogels can be programmed and fine-tuned by alterations in the structure of the building blocks [3,7]. Accordingly, the cargo (mostly drugs) can either be conjugated chemically to the nanogels or merely entrapped physically into its core [15], depending on the stimulus that is most feasible for utilization at the targeted site of disease.

Often multifunctional nanogels, which respond to a combination of more than one external signal, can also be fabricated for more site-specific response [48-51]. These stimuli are frequently disease- or organ-specific, since different pathological conditions are associated with changes in pH, temperature and redox balance or expression levels of certain biomolecules as compared to the normal physiological conditions [52]. The reversible crosslinks containing disulfide bonds that are stable in extracellular milieu but are cleaved in

the reductive intracellular environments due to the differences in the reductive potential between the extracellular and intracellular compartments, are successfully utilized in design of redox-responsive nanogels [12,49,53,54]. The nanogels with disulfide links are stable during circulation in the blood but labile while internalized in cells, thus, facilitating the release of the drug. Matyjaszewski et al. have prepared biodegradable nanogels with disulfide-functionalized dimethacrylate crosslinkers that degraded into individual polymeric chains in the presence of glutathione and enabled both the release of encapsulated cargos and the removal of the carriers [55]. Thayumanavan and coworkers reported the preparation of redox-sensitive nanogels based on random amphiphilic copolymer that contains hydrophilic oligoethyleneglycol (OEG) and hydrophobic pyridyldisulfide (PDS) units as side-chain functionalities [56]. Self-assembled nanostructures formed by this polymer were crosslinked by initiating a thiol-disulfide exchange reaction among the PDS groups using dithiothreitol (DTT). The size of the resulting nanogels can be tuned by varying the copolymer molecular weight, composition and concentration, while the extent of crosslinking can be controlled by the amount of added DTT. The release of the encapsulated doxorubicin (DOX) was triggered by glutathione treatment and the release rate can be tuned by adjusting the crosslinking density.

The group of Wang took advantage of the activity of bacterial lipases, which are abundant in microbial flora, to construct a nanogel for the on-demand release of antibiotics [52]. In this approach, the triple-layered nanogel contains a hydrophobic lipase-sensitive poly(ε-caprolactone) (PCL) interlayer between the crosslinked polyphosphoester core and the PEG shell. Prior to reaching sites of bacterial infection, the antibiotics are protected inside the polyphosphoester core and are not released due to the compacted PCL molecular fence. However, rapid drug release was observed in the presence of lipase or lipase-secreting bacteria.

In another scenario, the changes in the properties of nanogels can be caused by external signals in the form of radiation or magnetic field depending on the accessibility of the targeted site in the body [57,58]. An elegant design of near-infrared (NIR) light-responsive core-shell nanogels was recently reported by Kang et al. [59]. Au-Ag-based nanorods were used as templates for the synthesis of double-stranded oligonucleotide crosslinked polyacrylamide shell. When exposed to NIR-radiation, the heating generated by Au-Ag rods through photothermal conversion induced efficient thermal dehybridization of the linker oligonucleotides from their complementary sequences and led to a rapid gel-to-sol transition of the gel shell and effective payload release.

2.3 In vivo behavior

Nanogels are macromolecular systems specifically designed to achieve long circulation half-lives of their cargo *in vivo*, along with their ability to deliver this cargo at the desired site (Figure 1). To realize this, a nanogel, or any nanoparticulate system has to overcome many barriers, especially when administered via routes other than intravenous, like oral, intradermal, pulmonary, intraocular, etc. Depending on the route of administration, nanogels are designed specifically to overcome associated barriers and reach the circulation intact. Nanogels prolong circulation half-life of their cargo by 1) preventing their fast clearance

especially in the case of small molecules and 2) prevent quick degradation or metabolism which is more relevant for biomolecules. One of the most important obstacles to achieving prolonged circulation is opsonization of the nanogels followed by their clearance via organs of the MPS like liver and spleen, where they are taken up by the resident monocytes and macrophages [60]. PEGylation of the nanogel surface imparts them with 'stealth' properties by making the surface more hydrophilic, shielding a charge that the core might carry and establishing a steric hindrance for interaction with serum proteins, although this is highly dependent on the size of nanogel, its shape, molecular weight and surface density of the PEG used [61,62]. While PEGylation endows nanosystems with long circulation properties and reduces MPS uptake, eventually opsonization and macrophage clearance still occurs [63]. A number of studies have demonstrated that PEGylation of nanoparticles tends to shift their accumulation towards the spleen instead of the liver as compared to their non-PEGylated counterparts [64]. A unique feature that helps nanogels partially escape splenic filtration process is their softness and deformability. This is can be explained by an example in nature, namely erythrocytes, which in spite of having a size range in microns are easily able to pass through the splenic filtration bed that has a pore size of few hundred nanometers, due to their flexibility and deformability [62,65]. In fact, old RBCs are cleared from circulation mainly because they lose their flexibility. This biomimetic property of the nanogels can be highly advantageous for their *in vivo* application. For example, Lyon and colleagues have recently reported that soft spherical acrylamide-based nanogels deform and pass through membrane pores several times smaller than their hydrodynamic diameter under physiological pressures [66]. Also, varying the moduli of similar nanogels has been shown to affect their mechanism and rate of uptake in macrophages [67]. Merkel et al. reported that decreasing the modulus of microgel particles altered their biodistribution properties, allowing them to bypass several organs, such as the lung, that entrapped their more rigid counterparts, resulting in increasingly longer circulation times [68]. Convincing evidence for the prolonged circulation time of soft PEG-based hydrogel nanoparticles compared to hard nanogels of the same size was recently provided by Mitragori and coworkers [69]. The deformability of the nanogels can be modulated by varying the crosslink density within the particle matrix as well as by varying the size of the crosslinking moiety [70]. Incorporation of electrolyte moieties into the polymer network of hydrogel particles to increase the swelling ratio is another straightforward and quite efficient way to decrease the modulus [2]. However, the distribution of charged groups on the surface of a particle can accelerate the clearance of particle. To address this drawback, DeSimone's group has recently developed a strategy to generate highly-swollen polyelectrolyte gel particle with near-neutral charge while retaining charged group in the interior [71]. Fraction of nanogels that escapes clearance by the mechanisms discussed above is then distributed into various organs by the circulating blood. Nanogels are usually too big to pass through the tight junctions of the normal endothelium but can efficiently accumulate in solid tumors or inflamed tissues that have unique structural features such as defective leaky and loosely compacted vasculature and impaired lymphatic drainage leading to the well-characterized enhanced permeability and retention effect (EPR) [72,73]. In addition to EPR, bioconjugation of nanogels to targeting ligands allows directing them to specific receptors or molecules differentially overexpressed on the diseased cells/tissues thus improving their retention at the targeted site as well as facilitating their cellular uptake [74,75]. Various small molecules, peptides,

aptamers, antibodies or antibody fragments have been explored for targeted delivery of nanogels and other nanomedicines in tissue- or cell-specific manner [76]. Ligand-mediated targeting influences the overall biodistribution profile of the nanogels as compared to their nontargeted counterparts and the bias of distribution is towards to those tissues that have a high expression of the receptor. This can help avoid excess accumulation of the nanogels at off target sites and reduce the associated side effects.

After extravasation from the blood compartment, nanogels have to diffuse through the tissue matrix in the interstitial space [77] and reach the targeted cells, where they are internalized by a number of different endocytotic mechanisms, depending on the size, shape, softness, charge and other surface properties of the nanogels and the type of cells and receptor being targeted. Internalization of the nanogels can occur via more than one pathway, making it a highly complex process. But in general, endocytosis eventually confines the particles into intracellular vesicles, from where they are trafficked into endosomes and ultimately lysosomes. At each of these stages, nanogels are exposed to varying pH of endosomal/ lysosomal lumen, degrading enzymes or reducing environments, which are often utilized as stimuli for the release of cargo held within the nanogels. Nanogel carriers can also be designed to target specific intracellular organelles or escape them, depending on the type of cargo that they carry. For example, it is essential for the nanogels to undergo endosomal/ lysosomal escape so that the encapsulated siRNA or oligonucleotides can be released in their active form in the cytosol where they are supposed to show their therapeutic effect [11]. This has brought growing interest in the use of nanogels made of bioresponsive polymers to promote escape by osmotic effects, membrane binding or membrane fusion or using pH-sensitive or reducible crosslinkers to facilitate nanogel destabilization following internalization and enhance delivery efficiency [78-81]. Degradability of the nanogels is also essential to minimize toxicities associated with the accumulation of the carrier in the body.

3. Nanogels as a therapeutic drug carrier

Nanogels are highly swollen and can incorporate 30% wt. or more of biological molecules and drugs through electrostatic, van der Waals and/or hydrophobic interactions or covalent bonding with the polymer chains. These loading capacities are unusually high and exceed those of liposomes and polymeric micelles [3,82]. As a result of drug loading, the nanogels collapse forming stable nanoparticles, in which biological agent becomes entrapped. Introducing dispersing hydrophilic polymers (e.g., PEG) in a nanogel structure can prevent their aggregation. During the collapse of the drug-nanogel complex hydrophilic polymer chains become exposed at the surface and form a protective layer around the nanogel. The control and versatility of polymer chemistry allows designing a broad range of drug formulations and inclusion of multiple therapeutic cargos within the same nanogel carrier [27,83]. Stimuli-responsive drug release via temperature or pH-induced volume collapse can also be very attractive for drug delivery applications. The functionalization of the nanogel surface can further facilitate their selective accumulation in the target tissue or cells [84-86]. Development of nanogels that can carry, protect, target and release therapeutic agents in spatially and temporally controlled manner is actively ongoing and their rational design can provide a platform for multiple applications.

3.1 Nanogels for small therapeutic molecule delivery

Over the past few years, significant progress has been achieved in application of nanogels as a delivery carrier for small biologically active molecules. Nanogels can be a versatile platform for the incorporation of various small drug molecules through the combination of electrostatic and hydrophobic interactions as well as hydrogen-bond formation [87]. The swelling of nanogels in an aqueous environment allows for easy permeation of the cargos. The rational design of the nanogels might be an effective tool to tune the drug release rates, to affect carrier-cell interactions, and achieve desirable therapeutic effect of the drugs. One of the most important features of weakly-crosslinked polyelectrolyte nanogels is their ability to incorporate molecules of the opposite charge. For example, cationic crosslinked PEG-polyethyleneimine (PEG-PEI) nanogels were explored for immobilization of negatively charged biologically active compounds such as retinoic acid, indomethacin or valproic acid [87,88]. These drug formulations formed stable colloidal dispersions at physiological pH and ionic strength, could be lyophilized and then redispersed. Similar nanogels and complexation strategy has been successfully utilized for incorporation of various nucleoside analog 5′-triphosphates [89,90]. It was reported that these drug-loaded nanogels could improve the delivery of the active triphosphates of therapeutic nucleoside analogs into cancer cells and inhibited tumor growth in the mammary carcinoma animal model [90]. Recently, the same group demonstrated the significant advantage of active 5′-triphosphates of nucleoside reverse transcriptase inhibitors encapsulated in cationic nanogels over free drugs in the antiviral therapy of HIV-1 infection in the central nervous system (CNS) [91]. Our group utilized a controlled template synthesis of nanogels by polyion complexation and crosslinking of doubly hydrophilic block ionomers, such as PEG-*b*-poly(methacrylic acid) (PEG-*b*-PMA) [21,40,92]. The resulting nanogels have swollen cores of a crosslinked PMA network surrounded by a shell composed of PEG chains. This synthetic approach allows versatile control of the macroscopic properties of nanogels (size, degree of swelling, drug loading) by changing the number and the chemical structure of the crosslinks [92]. Notably, in contrast to many nanoparticles these anionic nanogels exhibited very low nonspecific adhesion to nontargeted surfaces, which can minimize their off target effect and facilitate target-specific delivery [93]. Such core-shell nanogels can incorporate very large amounts (up to 50% wt.) of weakly basic drug DOX through electrostatic coupling with carboxylic groups in the cores. DOX-loaded nanogels were stable for a prolonged period of time, exhibited noticeable pH-sensitive behavior with accelerated release of DOX in acidic environment due to the protonation and swelling of the nanogel crosslinked cores, and demonstrated cytotoxic activities in cancer cell lines [94]. Introduction of reversible crosslinks with disulfide bonds in the PMA ionic cores (cystamine was used as a biodegradable crosslinker) allowed developing nanogels that are degradable in the presence of the reducing agent (glutathione, cysteine), which in turn facilitated the release of the incorporated DOX [94]. This difference in the release kinetics led to considerable increase of cytotoxicity: degradable DOX-loaded nanogels displayed nearly six-fold higher cytotoxic activity than non-degradable nanogels. Shi et al. used PEG-poly(L-glutamic acid-*co*-L-cystine) copolymers to prepare nanogels stabilized by disulfide bridges and also demonstrated that DOX release was accelerated in intracellular reductive and acidic conditions [95]. DOX was loaded into these polypeptide-based nanogels, and an accelerated release was observed in glutathione monoester pretreated HeLa cells.

Anionic PEG-*b*-PMA nanogels were further used to encapsulate hydrophilic drug cisplatin through coordination interactions with COOH functionalities [40]. Cisplatin-loaded nanogels displayed pH-sensitive release of Pt(II) species in sustained manner that can be effectively controlled by adjusting the degree of crosslinking of the crosslinked cores. The released platinum species retained their activity and were able to form Pt adducts with nuclear DNA in the cancer cells [40]. It was shown that loading of cisplatin into the nanogels greatly improved drug therapeutic index by improving PK, enhancing tumor delivery, increasing antitumor efficacy, and mitigating the cisplatin-mediated nephrotoxicity in a mouse model of ovarian cancer [96]. When the same cispaltin-loaded nanogels were also decorated with targeting ligands (e.g. folate or LHRH peptide), tumor growth inhibition could be even further enhanced [85,86,97]. Peng et al. described dual pH- and temperature-responsive nanogels based on N-isopropylacrylamide, MAA, and PEG methylether methacrylate (NIPAAm-MAA-PEGMA) for the entrapment and release of cisplatin. It was shown that cisplatin release can be accelerated at acidic pH, and could be additionally controlled by the temperature change due to the deswelling behavior of these nanogels at the body temperature [98].

Small molecules usually contain only limited number of ionic groups, which are able to interact with oppositely charged nanogels, and complementary hydrophobic, hydrogen or coordination bonding between drug molecules and nanogels can further stabilize the electrostatic pairing [87]. Recently, our laboratory demonstrated that microenvironment formed by the hydrophobic domains in the nanogel ionic cores influences solubilization capacity and release characteristics of the nanogels [99]. Diblock copolymer, PEG-*b*-poly(L-glutamic acid), hydrophobically-modified with L-phenylalanine methyl ester moieties was used for the synthesis of nanogels with small size (ca. 70nm in diameter) and narrow particle size distribution. Stable DOX-loaded hybrid nanogels were prepared at high DOX capacity (30% wt.). It was shown that the release rates of DOX from hydrophobically-modified carriers were substantially less compared to nonmodified nanogels: a burst release of over 85% of the incorporated drug within 8h for nonmodified nanogels was observed while only ~20% of the incorporated DOX was released from hydrophobically-modified nanogels during the same period of time. These results suggested that intermolecular interactions in combination with more compact crosslinked core could account for the delayed and controlled release of DOX from hydrophobically-modified polyelectrolyte nanogels. It was also found that these DOX-loaded nanogels exhibited an improved antitumor efficacy compared to free DOX in an ovarian tumor xenograft mouse model.

Nanogels have also been explored as a carrier for poorly water-soluble drugs. Wang et al. introduced thermoresponsive nanogels based on chitosan–poly(NIPAAm-*co*-acrylamide) for the delivery of paclitaxel (PTX) [100]. Loading capacity of these nanogels for PTX was around 9% wt. and drug-nanogel formulation showed very good colloidal stability. This nanogel released PTX in a temperature-dependent manner wherein significantly faster drug release was achieved at higher temperature. Moreover, PTX-loaded nanogels demonstrated improved antitumor efficacy in mice bearing HT-29 colon carcinoma tumors after intravenous administration. In another study by Gref and coworkers, hydrophobic molecule benzophenone (widely used as sunscreen agent) was solubilized into nanogels formed spontaneously upon the association of a lauryl-modified dextran and a β-cyclodextrin

polymer in aqueous media [101]. The highest benzophenone loadings (about 2.5 % wt.) were obtained by solubilizing it in both polymer solutions before mixing them to form nanogels. Such nanogel-based formulations present a compelling future opportunity for the application in the cosmetic field as sun screen carriers prepared by a simple "green" technology. The use of nanogels for the development of long-lasting formulations of local anesthetics has recently been reviewed by Tan et al [102]. Eckmann and colleagues explored biocompatible physically crosslinked hybrid nanogels consisting of a partially denatured lysozyme cores and dextran shells for the local delivery of dexamethasone to alleviate acute pulmonary inflammation [103]. To target the pulmonary vasculature, nanogels were coated with antibodies directed to endothelial determinant, Intercellular Adhesion Molecule-1 (ICAM). The synthesized ICAM- targeted nanogels were loaded with dexamethasone at 5% wt. *In vivo* studies in animal model of LPS-induced lung injury showed that nanogels targeted to lungs succeeded in delivering encapsulated dexamethasone, as was indicated by drastic reduction in pulmonary vasculature inflammation to levels found in naïve mice. In other work, similar dextran-lysozyme nanogels were utilized as scaffolds for the *in situ* synthesis of silver nanoparticles [104,105]. Such hybrid nanogels exhibited bactericidal properties towards *E. coli* and bacteriostatic properties towards *S. aureus*, and their antibacterial activity can be tuned by varying lysozyme content. The tunability of the hybrid nanogels makes it possible to optimize release of the bactericidal agent for specific clinical use and type of infection. Nagasaki's group used the protonated crosslinked poly(2-[N,N-diethylamino]ethyl methacrylate) (PEAMA) cores of the stimuli-responsive PEGylated nanogels for the synthesis of gold nanoparticles without any reducing agents ("gold nanogels") [106]. It was shown that the tertiary amino groups in the PEAMA gel core play a crucial role in the reduction of the Au(III) ions (nanoreactor) as well as the immobilization of the resulting gold nanoparticles (nanomatrix) to attain large payloads of gold nanoparticles. The resulting gold-containing nanogels showed a remarkable photothermal efficacy in response to laser-irradiation resulting in selective cytotoxicity in cancer cells. In other studies, a potential of the similar constructs to enhance cancer cell radiosensitivity was reported [107].

3.2 Nanogels for oligonucleotide delivery

Therapeutic oligonucleotides (ONs) including antisense oligodeoxynucleotides (ODNs), small interfering RNAs (siRNAs), and the more recently discovered micro RNAs (miRNAs) designed for targeted inhibition of specific mRNA sequences are of emerging interest for the treatment and diagnosis of cancer [108-110], neurodegenerative disorders [111-113] and lethal viral infections [114-116]. Some oligonucleotide-based therapies have already achieved significant success in clinical trials [117,118]. However, the delivery of ONs into targeted cells remains a key challenge to realizing their full therapeutic potential because ONs are negatively charged, hydrophilic molecules that are unable to penetrate cell membranes on their own, can be degraded by endogenous nucleases and can stimulate innate immune system. Thus, ONs require a delivery vehicle to bring them to site of action without adverse effects. Cationic nanogels have emerged as promising new class of nanomaterials to address the challenges of *in vivo* ONs delivery [84]. Composed of weakly crosslinked hydrophilic polymer chains, nanogels have a high degree of porosity that permits the

effective encapsulation of macromolecular therapeutics, which usually cannot be achieved with conventional nanocarriers.

In one of the first reports, Vinogradov et al. exploited cationic PEG-PEI nanogels as potential carriers for antisense phosphorothioate ODN specific to human mdr1 gene [119]. Significant enhancement of antisense inhibition of Pgp as a result of incorporation of ODN in the nanogel was observed. Modification of ODN-loaded nanogels with transferrin targeting moieties additionally increased the mdr1-inhibitory effects and was shown to facilitate transport of the loaded nanogels across the blood-brain barrier [84]. Subsequently, DeSimone and coworkers used inverse miniemulsion polymerization of 2-acryloxyethyltrimethylammonium chloride and 2-hydroxyethylacrylate with PEG-diacrylate crosslinker to synthesize biocompatible nanogels with controlled size, morphology, and composition capable of forming stable ODN complexes and enhancing cellular delivery of ODNs in vitro [16]. Nowadays, a variety of cationic nanogel particles have been actively adapted to deliver siRNA molecule [81,120-128]. For example, Anderson and coworkers synthesized a library of 1,536 structurally distinct core-shell nanogels for siRNA complexation and delivery with great variability in the chemical nature of the protonizable amine-based core and a shell with variation in polymer length and chemical properties [129]. The evaluation of this library revealed that internalization and/or complexation alone is not sufficient for silencing and that certain chemical functionalities with potential buffering capacity may be advantageous for nanogel-based delivery. Chemical modifications of siRNA may also be used to enhance the transfection efficiency of these nanocarriers and to impart greater stability to their nucleic acid cargoes. The recent report by Zentel group [78] also emphasized that size-dependent uptake and intracellular distribution mechanism of siRNA-loaded cationic nanogels may be essential for tuning their knockdown potential. The authors used two sets of well-defined nanogels based on amphiphilic block copolymers of pentafluorophenyl methacrylate and tri(ethylene glycol) methyl ether methacrylate crosslinked by spermine with diameters of 40 nm and 100 nm. Both formulations show similar physicochemical properties, loading efficiencies and release capabilities of siRNA. However, only the small sized anti-luciferase siRNA-loaded nanogels with diameters of about 40 nm were able to avoid acidic compartments of endolysosomal uptake pathway and induce gene knockdown, while the 100 nm-sized nanogels did not affect the gene expression of luciferase at all. The reader is referred to more detailed review articles that summarize the key properties of the cationic nanogels that enable successful in vivo delivery of various nucleic acids [130].

3.3 Nanogels for delivery of protein therapeutics

Owing to the ability of nanogels to encapsulate high amounts of biomacromolecules and prevent them from degradation, they have been also widely explored for the delivery of proteins and peptides. In their pioneering work, Akiyoshi et al. reported that the nanogel of self-assembled cholesterol-modified pullulan (CHP) forms a complex with various kinds of proteins spontaneously, primarily through hydrophobic interactions [131,132]. The amount of protein complexed by such nanogels depends on the molecular weight and hydrophobicity of the protein. Complexation drastically suppressed the thermal denaturation and subsequent aggregation of proteins as well as protected them from enzymatic degradation. This platform

was further extended by cationization of the polysaccharide-based nanogels to utilize both hydrophobic and electrostatic interactions for effective protein trapping and to enhance cellular internalization of the protein-loaded carriers [10]. However, one of the drawbacks of such delivery systems is the destabilization of the nanogel–protein complex *in vivo* in the presence of high protein concentrations. To preserve the long-term stability of such complexes, the same group developed the raspberry-like assembly of nanogels with narrow size distribution (40-120 nm size) by crosslinking acrylate-group-modified CHP with thiol-modified tetra-armed PEG [133]. These nanogel assemblies showed high encapsulation efficiency for interleukin-12 (IL-12) and were able to maintain steady plasma IL-12 level in mice up to 72 h following subcutaneous administration. Nagahama et al. demonstrated that hybrid self-assembled nanogels based on poly-(L-lactide)-grafted dextran enable sustained release of the entrapped lysozyme in its active form for a week without initial burst release at physiological conditions [134]. Thienen et al. developed biodegradable lipid-coated dextran nanogels by UV polymerization of dextran derivatized with 2-hydroxyethyl methacrylate moieties using liposomes as a nanoscaled reactor [135]. Proteins can be loaded into the nanogel cores with high efficiency during the polymerization process and their release can be controlled by crosslinking density of the gel matrix. The application of polysaccharide-based nanogels for delivering proteins and other macromolecular therapeutics has recently been summarized in a review article [136]. Matyjaszewski and coworkers demonstrated that application of atom transfer radical polymerization (ATRP) enabled the synthesis of functionalized nanogels with a uniform network that is capable of efficient encapsulation of proteins *in situ* [137]. Similarly, the addition of cytochrome C during the inverse emulsion reversible addition fragmentation chain transfer (RAFT) polymerization of *N*-(2-hydroxypropyl)methacrylamide and *N,N´*-bis(acryloyl)cystamine also showed high loading efficiency (73 % wt.) of the protein in the resulting nanogels, which were shown to release their payload under reductive conditions [138]. In another interesting work reported by Chen et al. RAFT polymerization was used to prepare disulfide crosslinked nanogels based on PEG-*b*-poly(2-(hydroxyethyl) methacrylate-*co*-acryloyl carbonate) for loading (~50 % wt.) and triggered intracellular release of proteins [139]. The *in vitro* release studies showed that release of fluorescently-labeled cytochrome C was minimal under physiological conditions while complete release of the protein from nanogel was observed in the presence of 10 mM dithiothreitol over 22 h. Cytochrome C-loaded reduction-sensitive nanogels demonstrated apparently better apoptotic activity than free cytochrome C and reduction-insensitive controls. Shi et al. developed acid-labile nanogels via inverse emulsion polymerization of N-vinylformamide in the presence of a ketal-containing crosslinker. The loading capacity of these nanogels for lysozyme was ~60% wt. and approximately 95% of lysozyme encapsulated in nanogels released over 3 hours at pH 5.8 compared to only ~15% released at pH 7.4. Notably, released lysozyme retained about 50% of the original activity. Although the better loading efficiency is a significant advantage of the aforementioned *in situ* loading technologies, the retention of activity, structural identity, and stability of protein after encapsulation are basic concerns in development of biopharmaceutical products.

Polymeric nanogels have also found an application in the formulation of a new generation of therapeutic and preventive vaccines. Intrinsic properties of polymeric nanogels, such as

material chemistry, size and shape, surface charge, and hydrophobicity or hydrophilicity, may be determining factors in designing the induced immune response. These materials can thus work as synthetic adjuvants, which can also be conjugated with immunostimulants. Furthermore, nanogels having a size of 50 nm or less were found to be quite effective in delivery across the hydrogel-like network of mucus [140] and it has been reported that in the case of nanogels for vaccine delivery, the type and intensity of immune response can be modulated by altering the size of the nanogels [141-143]. In a very interesting report published by Akiyoshi and Kiyono group, cationic cholesterol-bearing pullulan (cCHP) nanogels were explored as protein-delivery system for adjuvant-free intranasal vaccines [14]. As shown in Figure 2, they demonstrated that intranasally administered cCHP nanogel loaded with a non-toxic subunit fragment of Clostridium botulinum type-A neurotoxin BoHc/A (cCHP–BoHc/A) adhered to the nasal epithelium and BoHc/A was effectively taken up by mucosal dendritic cells after its release from the cCHP nanogel. Importantly, intranasally-administered cCHP–BoHc/A did not accumulate in the olfactory bulbs or brain, diminishing safety concerns about the potential dissemination of vaccine antigens to the CNS. Moreover, intranasally immunized tetanus toxoid with cCHP nanogel induced strong tetanus-toxoid-specific systemic and mucosal immune responses. The CHP platform has also been tested in Phase 1 clinical trials as carrier for HER-2 antigen in patients with therapy-refractory HER-2 positive cancers [144] as well as for delivery of NY-ESO-1 antigen in esophageal cancer and malignant melanoma patients [145,146]. Collectively, the results from several clinical trials performed using the CHP-based antitumor vaccines demonstrated the safety of the vaccines after repeated subcutaneous administration as well as document their success in inducing both antigen-specific CD4+ and CD8+ T-cell response along with humoral immunity. According to a more recent report, a MAGE-A4-specific humoral immune response was observed in four patients out of fifteen vaccinated with MAGE-4A protein encapsulated in CHP nanogel [147]. These patients showed prolonged overall survival, significantly longer than that of patients without a MAGE-A4 antibody response after vaccination. We encourage the readers to refer to a review by Ferreira et al. [141] and the latest review by Akiyoshi [148] for the details about the application of nanogels as vaccine carriers.

3.4 Nanogels for combination drug delivery

Drug combinations directed simultaneously at multiple pharmacological targets have the potential to dramatically improve the response to treatment and are already a standard clinical practice in the treatment of cancer and infectious diseases [149]. However, the difference in pharmacokinetic and pharmacodynamic profiles among diverse drug molecules makes dosing and scheduling optimization very challenging. Combining drugs in one delivery carrier is a well-suited strategy for controlling the pharmacokinetics and co-delivery of the desired drug ratio *in vivo*, and a variety of nanoscale carriers, including nanogels, have been investigated in terms of their ability to deliver multiple drugs [27,83,150,151]. As highlighted in previous sections, the nanogel structure can be readily adjusted to integrate features of different materials and, thus, offer advantages for combinatorial encapsulation of drugs with varying physicochemical properties such as small molecules, proteins and nucleic acids. Fahmy and coworkers developed liposomal nanogels of drug-complexed cyclodextrins and cytokine-encapsulating biodegradable polymers that can deliver small hydrophobic

molecular TGF-β inhibitor and water-soluble protein cytokine (IL-2) in a sustained fashion to the tumor microenvironment [150]. They demonstrated that synergistic effects of simultaneously delivered IL-2 and TGF-β inhibitor on activation of the innate arm of the immune system led to delayed tumor growth and enhanced survival of melanoma tumor-bearing mice after systemic administration. In line with the challenge of delivering drugs with different properties, our group developed multi-compartment core-shell nanogels based on hybrid triblock copolymers, PEG- poly(L-glutamic acid)- poly(L-phenylalanine) (PEG–PGlu–PPhe). Such nanogels have a central hydrophobic core formed by PPhe chains, a crosslinked anionic layer of PGlu chains, and an outer shell composed of PEG chains [27]. These hybrid biodegradable nanogels can entrap considerable amount of drugs with very different physical properties such as hydrophilic cisplatin (15 % wt. loading) and hydrophobic paclitaxel (9% wt.). Binary drug combination in nanogels exhibited synergistic cytotoxicity against human ovarian A2780 cancer cells and exerted a superior antitumor activity in cancer xenograft models *in vivo* as compared to individual drug-loaded nanogels or free drugs. The benefits of synchronized co-delivery of the platinum-taxane drug combination via single carrier can be further enhanced by targeting nanogels to folate receptor, which are overexpressed in most ovarian cancers [97]. In another study, we reported an efficient co-encapsulation of DOX and 17-allylaminodemethoxygeldanamycin (17-AAG) into PEG-PGlu nanogels with multiple hydrophobic domains that are located within the crosslinked polyion PGlu core [83]. Dual drug-loaded nanogels displayed potent cytotoxicity in a breast cancer cell panel and exerted selective synergistic anticancer activity against ErbB2-overexpressing breast cancer cell lines. This synergistic effect was attributed to the action of 17-AAG, HSP90 inhibitor, which induces degradation of many of the proteins required for DNA- damage response as well as attenuates hyperactive ErbB2 downstream oncogenic signaling, thereby rendering cancer cells more vulnerable to the cytotoxic effects of DOX. Consistent with the *in vitro* findings, combination treatment with nanogels exhibited superior antitumor efficacy, both in terms of tumor inhibition and survival, in an ErbB2-driven xenograft model compared to the cocktail of free drugs at equivalent drug concentrations.

Akiyoshi and colleagues evaluated nanogels based on acrylate group–modified CHP for co-delivery of prostaglandin E2 receptor–specific agonist EP4A (small molecule) in combination with bone morphogenic proteins (BMP-2) for bone regeneration [151]. In this study, EP4A-containing nanogels and BMP-2-containing nanogels were crosslinked with thiol-bearing PEG to obtain disc-like scaffolds for the implantation into large bone defects. Combination treatment with EP4A and low-dose BMP-2 efficiently activated bone cells to regenerate calvarial bone by forming both outer and inner cortical plates as well as bone marrow tissue and resulted in the formation of enough bone to cover the defects in calvarial bone. In another study, cationic physically-crosslinked nanogels composed of a hexadecyl group-bearing cycloamylose and spermine-modified cycloamylose to complex and co-deliver plasmid DNA along with phospholipaseA2 (PLA$_2$) [152]. As a lipolytic enzyme, PLA$_2$ catalyzes hydrolysis of a variety of different phospholipids. Thus, being delivered into endosomes by nanogel it could disrupt the lipid membrane and subsequently trigger the release of the co-encapsulated DNA into the cytoplasm. Transfection experiments confirmed that DNA expression level was enhanced when complexed with PLA$_2$. Similarly, pDNA and

proteins were successfully co-encapsulated using pH- and temperature-sensitive carbohydrate-based nanogels [79]. The nanogels had a core-shell structure with a crosslinked hydrophobic core that could be loaded with proteins and the shell contained carbohydrate residues that allowed for the complexation of DNA. These nanogels were capable of loading larger-than-normal amounts of cargo by using a heating and cooling cycle. Altogether, these studies may open new avenues for the development of the carrier-mediated combination therapies.

4. Nanogels in diagnostics and imaging

Structural versatility of nanogels, high water content, fluid-like transport properties and biocompatibility make them ideal carriers for various imaging probes and contrast agents. Introduction of multiple functional groups either in the interior or on the surface of nanogels allows for incorporation/conjugation of multiple dyes, reporter molecules or inorganic nanoparticles. Encapsulation of magnetic nanoparticles such as iron oxide into crosslinked nanogels has been shown to confer both colloidal stability and better sensitivity than when these agents are administered as non-encapsulated entities [153,154]. Nanogels allow for the encapsulation of a large cargo of the magnetic nanoparticles, which can lead to generation of much stronger local magnetic fields due to the cluster effect [154-157]. Moreover, the hydrogel coating further increases the relaxivities by lowering the diffusion coefficient of water near the particles and prolonging the interaction between the water protons and the high magnetic fields at the surface of the particle. The extent of reduction in the diffusivity of the water molecules depends on the thickness of the gel coating around the magnetic particle [156-159]. This could allow for partial control over the relaxation times by manipulating the swelling/deswelling transition of the nanogel matrix [157]. Interestingly, Okada et al. reported that nanogels consisting of crosslinked PMA can function as magnetic resonance imaging (MRI) pH-sensors without the need of any inorganic paramagnetic material [160]. The shrinking of pH-sensitive nanogel at acidic pH induces more rigid configurations and slower rotational motions of the polymer chains than in the swollen state thereby leading to shorter transverse relaxation time because the mobility of bound water molecules is highly restricted. Another factor that influences the relaxation time of contrast agents like Gd chelates is the freedom of tumbling motion [155]. One of the best methods to restrict tumbling is by conjugation of the contrast agent to a macromolecular system, and nanogels serve as the perfect hydrophilic platform with flexible loading capacity for entrapment for such agents, which was shown to result in a further relaxivity enhancement. [156,161,162]. Gd^{3+} ion is toxic and it is clinically administered in the form of chelates. However, even the chelates have the potential to show toxicity due to transmetallation reactions, which involve the displacement of the chelated metal ion by another competing ion. It has been reported that diethylenetriaminepentaacetic acid (DTPA)-based nanogels are more inert to transmetallation reactions than free chelates [163]. Encapsulation into nanogels also helps in reducing the toxicity associated with inorganic nanoparticles [164-168]. Furthermore, it has been shown that the electronic and optical properties of gold and silver nanoparticles are highly dependent on their size and shape [169-171] and encapsulation of such nanoparticles into nanogels becomes essential not only for their colloidal stability but also for consistency of the imaging purpose. As in the case of contrast agents, the swelling/

deswelling of the surrounding nanogel network in response to changes in the microenvironment can reversibly alter the interparticle distance between gold nanoparticles leading to a corresponding change in their optical properties. This can impart tissue specificity to the imaging function to a certain extent [171,172].

4.1 Nanogels as MR contrast agents

Small molecule MR contrast agents based on gadolinium (Gd) and manganese (Mn) are all rapidly cleared from the body and suffer from toxicity issues. [173,174]. To overcome these challenges, Soleimani et al. prepared a nanogel by copolymerization of PEGMA, N-(2-aminoethyl)methacrylamide hydrochloride, and the crosslinker ethylene glycol dimethacrylate under free radical conditions [156]. The reaction conditions were optimized to obtain nanogels with a size on the order of 10 nm. An isothiocyanate derivative of the chelator DTPA was then conjugated to nanogel for the insertion of Gd (III). The nanogel contrast agent exhibited ~ 5-fold enhancement in relaxivity compared to clinically used Gd(III)–DTPA (Magenvist). Signal enhancement, however in lesser extent, was also observed for non-crosslinked polymer constructs containing similar amount of Gd-chelates. The modeling of nuclear magnetic resonance dispersion data suggested that the enhancement in relaxivity for nanogel vs linear polymer arise from the constraint on polymer chain motion imparted by the crosslinking that leads to additional slowing of the chelates' molecular tumbling rate. Alternatively to post polymerization functionalization of nanogel with contrast agents, Gd-chelates can be incorporated into nanogels within the crosslinking moieties or Gd^{3+} ions can be used as metal crosslinkers themselves. The later strategy was utilized by Kim and coworkers to prepare Gd-coordinated nanogels by crosslinking of branched PEI with Gd^{3+} ions in inverse microemulsion followed by surface functionalization with PEG chains in order to increase the blood circulation time [175]. It was shown that polydentate chelation by PEI is robust enough to minimize the liberation of toxic Gd^{3+} ions under the physiological conditions. Interestingly, the determined longitudinal T_1 relaxivity values for Gd-PEI nanogels were lower than that for Gd-DTPA chelates. The reduced relaxivity was attributed to the large nanogel core size with a small surface-to-volume ratio, which hinders the direct Gd^{3+}-water contact that is prerequisite for influencing the longitudinal relaxation. In contrast to other T_1-enhancing Gd complexes, Gd-PEI nanogels showed the capability of enhancing negative T_2 contrast, which was assigned by high density of the Gd^{3+} ions in the cores of the nanogels. Almutairi's group reported Gd-chelating polyacrylamide nanogels prepared by radical polymerization of acrylamide with metal chelating crosslinking agents in an inverse emulsion [163]. The resulting crosslinked nanogels had a size in the range of 50 – 85 nm, were stable against transmetallation, and exhibited a substantial (3 to 4-fold) increase in relaxivity. To further improve nanogels biocompatibility, the same group utilized nanogels formulated from cholesterol- and acryloyl-modified pullulan (CHPOA). The surface acryloyl groups of CHPOA were chemically crosslinked with Gd-chelating 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) crosslinker. At a magnetic field of 1.41 T, T_1 relaxivity of these Gd-chelating pullulan nanogels was measured to be 24.1 ± 0.6 mM^{-1} s^{-1}, which corresponds to 6-fold enhancement as compared to low-molecular weight chelates, Magnevist and Dotarem. The reported value was also the highest relaxivity among all other nanogel-based contrast agents. MRI of tumor-bearing mice showed that Gd-CHPOA provides great contrast in

tumors with exceptionally high signal-to-background ratio. This allowed for discriminative and accurate tumor identification along with determination of the surgical margin. Importantly, the long-term accumulation of Gd-CHPOA in organs of the MPS like liver and spleen did not cause any damage or toxicity up to three months after injection.

Superparamagnetic iron oxide (SPIO) nanoparticles function as T_2-weighted MR contrast agents and have exerted a large impact on the field of molecular and bioimaging. Their nonspecific uptake by MPS cells found clinical applications for imaging liver tumors [176] and lymph nodes [177]. SPIO nanoparticles' magnetic properties can be manipulated by changes in size and surface chemistry. Specifically, the local T_2 proton relaxivity of SPIO nanoparticles can be modified via interfacial surface chemistry to induce aggregation or dispersion in the colloid, thus providing control of the ultimate nanoparticle aggregate size and magnetic properties [178]. Katagiri et al. reported a synthetic approach for the *in situ* formation and immobilization of SPIO nanoparticle aggregates in nanogels [153]. Iron ions were complexed within the pre-heated network of physically crosslinked CHP nanogels and oxidized by addition of ammonia water solutions. This strategy minimized homogeneous nucleation outside of nanogels, and the resulting hybrid nanogels had a size similar to that of the empty nanogels (~ 30 nm). Essentially, the empty nanogels acted as nanoreactors wherein the volume in which the reaction could occur was confined by the nanogel network. However, the crystallinity of iron oxide particles synthesized in nanogels was relatively low, and will not translate into high saturation magnetization; therefore, these hybrid nanogels are not suitable for use as MR contrast agent. To overcome this drawback, the preformed oleate-coated iron oxide nanoparticles with size of 12 nm and high crystallinity were complexed with nanogels through hydrophobic interactions with cholesterol groups in the nanogels [179]. The resulting hybrid showed high colloidal stability and displayed higher relaxivity than existing contrast agent Resovist. In addition, the hybrid nanogel generated heat following irradiation with alternating magnetic field, which indicates that these materials can also be suitable for magnetic hyperthermia therapy.

Recently, Wang et al. described a nanogel-based system co-loaded with both T_1 and T_2 relaxivity contrast agents, manganese oxide and SPIO nanoparticles, since the use of dual contrast agents can potentially allow for higher accuracy of imaging. These agents cannot be simply mixed in a single system, since in close proximity they would lead to mutual nullification of the signal, as the water molecules can interact freely with both the agents. This problem, however, was solved by the use of stimuli-responsive chitosan-based nanogels, which release Mn^{2+} ions only in the acidic pH inside the tumor cells while at physiological pH in normal cells the system remains silenced, thus improving specificity of imaging. This technique can be potentially useful for detection of small tumors with better accuracy than the existing single agent probes [180].

Nagasaki's group developed a unique tumor specific nanogel-based probe for ^{19}F magnetic resonance spectroscopic imaging (^{19}F MRS/I) [181]. ^{19}F MRS/I has been recognized as a powerful methodology due to high MR sensitivity of ^{19}F and low background noise which is attributed to the absence of endogenous ^{19}F in the body. The ^{19}F MRS/I of tumors can be further improved using a probe that attenuates the ^{19}F MR signal outside of the tumor and switch on the signal inside the tumor. To this end, a pH-responsive nanogel consisting of a

crosslinked PEAMA-*co*-poly(2,2,2-trifluoroethyl methacrylate) gel core and PEG shells was synthesized. These nanogels showed remarkable activation of ^{19}F MR signals upon change of pH from 7.4 to pH 6.5, which corresponds to the extracellular pH of tumor tissues (7.0 – 6.5). Such enhancement was attributed to the volume phase transition of the gel core at acidic pH that leads to an increase in molecular motion of the ^{19}F atoms and longer T_2 relaxation times.

4.2 Nanogels for PET imaging

Polyacrylamide-based nanogel crosslinked with polydentate chelating ligands that were developed by Allmutairi and coworkers can be also used as a scaffold for metal radionuclides to obtain PET radiotracers [182]. Various crosslinkers based on DTPA, DOTA or 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) were synthesized to optimize the chelation stability of nanogels. Experiments in mouse serum indicated that NOTA-based nanogels retained ^{64}Cu most stably, with very little transchelation in comparison with the other two crosslinkers. ^{64}Cu-DOTA-crosslinked nanogels showed high accumulation in the tumor as well as lower signal in the liver and spleen compared to DOTA-based nanogels. In some cases, the accumulation of ^{64}Cu-DOTA in metastases was even higher than in the primary subcutaneous tumor. These promising data suggest that nanogels incorporating metal-chelating crosslinks can be useful as PET agents in cancer diagnosis and therapy monitoring. An advantage of such systems is that the radioisotope can be easily incorporated into preformed nanogels immediately before their clinical application.

Singh et al. designed reduction-sensitive crosslinked nanogels containing chelating groups for ^{68}Ga and other trivalent metals [183]. Self-assembly of amphiphilic, partially thiolated star-shaped poly(ethylene oxide-*stat*-polypropylene oxide) copolymers was used for the synthesis of the nanogels. The thiol chemistry was used for both crosslinking and derivatization for radiolabeling. It was shown that ^{68}Ga can be chelated very efficiently with high radiochemical yields. However, the size of the resulting nanogels (290 ± 50 nm) may not provide optimal circulation time and tumor uptake. *In vivo* PET imaging of ^{68}Ga-labeled nanogels has not yet been reported.

4.3 Nanogels for optical imaging

In vivo fluorescence-based optical imaging is most effective by using agents that emit in the NIR region (> 700 nm) due to minimal auto-fluorescence from the tissues in this wavelength range, and deep tissue penetration of excitation light [184,185]. Among available NIR probes, only indocyanin green (ICG) has been approved for clinical imaging applications. ICG, however, suffers from several limitations such as low quantum yield, relatively short circulation half-life, degradation in aqueous media and self-quenching above a certain concentration, and nonspecific interaction with various proteins in blood plasma, which alter its fluorescence emission properties [186].

Nanogels have been employed to overcome the above shortcomings of the dye in order to extend its potential as a NIR imaging agent. In fact, some of these limitations were used as advantages to design systems that would be activated specifically in the targeted cell population. Park et al. designed cancer cell-targeted nanogels containing ICG as the reporter

molecule that were non-fluorescent in the normal cells. A pH sensitive polymer, poly(β-amino ester) (PBAE) and CD44 receptor-targeting hyaluronic acid were used to form the nanogels which entrapped ICG. Under normal physiological conditions, the ICG fluorescence was quenched in the intact nanogels. Once ICG-loaded nanogel is internalized by receptor-mediated endocytosis, PBAE is degraded in the acidic environment of endosomes and lysosomes, gradually releasing ICG, thus generating a fluorescence signal [187]. In a similar vein, hyaluronic acid was used to prepare nanogels that could be used for monitoring hyaluronidase activity *in vivo*, as it is associated with tumor metastasis and angiogenesis. Shell crosslinked nanogels were prepared from hyaluronic acid via a reducible covalent linkage, which could incorporate ICG derivatives [188]. In the absence of the enzyme, HA-based nanogels had negligible fluorescence as compared to free ICG due to self-quenching effect. However, in the presence of the enzyme, the nanogel structure was disturbed and a strong fluorescence signal was generated. This was illustrated both *in vitro* and *in vivo* after intradermal injection of the nanogels in the forepaw of mice. Nanogels showed a strong fluorescence signal 5 mins after injection of hyaluronidase into the forepaw, indicating that this system could be used as hyaluronidase-specific probe. NIR imaging is also useful for detecting the cancer metastasis to regional lymph nodes, particularly the sentinel lymph node (SLN). Biopsy of SLN is usually done during tumor resection surgeries to determine whether metastasis has occurred, for which visualization of these lymph nodes is necessary during the surgical procedure. Small molecule dyes used clinically for this purpose suffer from the drawbacks of rapid lymphatic clearance and spreading to the entire lymphatic system and can therefore give false-positive results. Dextran-poly(acrylic acid) nanogels (DNG) were synthesized via self-assembly assisted method which involved hydrogen bonding between hydroxyl groups of dextran and carboxyl groups of poly(acrylic acid) [189]. The additional free carboxyl groups of poly(acrylic acid) served as a platform for the conjugation of amine-containing dyes. As a model agent, green 5-aminofluorescein was conjugated to obtain fluorescent DNG, which were found to drain via the lymphatic vessels and into SLN within a minute of being injected intradermally, and the fluorescence signal peaked around 12 h and lasted for 60 hours. This extended imaging window is well-suited for surgical applications wherein the visualization of lymph nodes is required. Another nanogel-based system designed for the same application has been described by Noh et al. [190]. IRDye800 containing nanogels composed of cholesterol-modified pullulan (NIR-CHP) had hydrodynamic diameters of about 30 nm, which is considered optimal for lymph node uptake (Figure 3). NIR-CHP probes exhibited enhanced photostability, and could accumulate and retain in the SLN after intradermal injection. The NIR-CHP were present at qualitatively consistent levels in the lymph node even at 48 h post injection, whereas the IRDye800 was not visibly present in the lymph nodes at this time point. In another study, the utility of NIR-CHP probes for SLN mapping was evaluated in large animal models [191]. The NIR-CHP signal intensity was relatively small at the injection site and limited to only one sentinel node with no spreading to consecutive distal lymph nodes. These characteristics of NIR-CHP make them useful as imaging contrast agents in sentinel node navigation surgery.

In recent years, there has also been a considerable interest in the development of optical probes based on nanogels and inorganic nanoparticles such as quantum dots and gold

nanoparticles. The reader is referred to some excellent review articles on the design of such hybrid nanogels and their application for optical sensing and bioimaging [165,192].

4.4 Nanogels for multimodal imaging agents

While a number of imaging techniques can be employed to obtain detailed information regarding various organs and tissues of the body, each imaging modality has limitations of either sensitivity or resolution. Therefore, the integration of several imaging agents with different properties into multifunctional nanoparticles may provide precise information about the existing pathological conditions through synergetic multimodal imaging. [193]. Due to their unique multifunctionality, large surface area, and structural diversity, nanogels are capable of loading more than one imaging/contrast agent within the same carrier that can help realize this objective.

For example, some of optical imaging complications associated with poor spatial resolution and lack of anatomical reference can possibly be mitigated when combined with other high resolution anatomical imaging modalities such as MRI. This combination was employed to define the glioma margins using targeted pH/temperature sensitive nanogels based on poly(NIPAAm-*co*-acrylic acid). Magnetic SPIO nanoparticles were incorporated into nanogel network during emulsion polymerization followed by conjugation with Cy5.5-labled lactoferrin as an optical probe and an effective targeting ligand for glioma [194,195]. The LCST of the nanogels at physiological pH of 7.4 was 40°C, making the nanogels swollen and hydrophilic, which could prolong the blood circulation time. In the acidic environment of tumor tissues (pH 6.8), the LCST is lowered to 34 °C leading to collapse of the nanogels, which could in turn potentiate their accumulation in tumor tissue and internalization by tumor cells. The authors reported very accurate signal localization and correlation of *in vivo* results to *ex vivo* images of excised brain (Figure 4) which was attributed to active targeting ability of the lactoferrin and passive targeting ability enhancement caused by the pH/temperature sensitivities of the nanogels [196].

Another multi-imaging study using nanogel-based dual MRI and optical imaging probe was reported by Lim et al. Core-shell nanogels containing Gd^{+3}-coordinated PEI core and PEG shell were functionalized with a NIR dye, Cy5.5 and evaluated in SCC7 tumor-bearing mice [175]. Although no targeting group was attached to the nanogels, tumors displayed strong NIR fluorescence signals up to 1 day post injection. Notably, the amount of injected nanogels corresponded to a much lower dose of contrast agent (38 μmol Gd/kg) than the standard dose of Gd-DTPA (100 μmol Gd/kg) and no notable contrast enhancement was observed in T_1-weighted images. However, a significant signal darkening was seen in certain regions of the tumor at 2 h post-injection, visualizing the intratumor structure at high spatial resolution, indicating that these nanogels could act as negative contrast enhancers (see section 4.1) for tumor imaging with the added advantage of fluorescence imaging [175].

Nanogels can also be designed to have self-fluorescence properties, as demonstrated by Kim et al. [197] using oppositely charged polyelectrolytes to form the gel network within which $MnFe_2O_4$ nanoparticles were encapsulated. Negatively charged poly(γ-glutamic acid) was condensed with polylysine (PLL) in the presence of $MnFe_2O_4$. The free amino groups of PLL were then crosslinked with glutaraldehyde, which also involved formation of a self-

fluorescent carbon–nitrogen double bond (C=N) and carbon-carbon double bond (C=C). These nanogels were finally surface-grafted with PEG. These dual-modality nanoprobes showed high performance in the labeling and monitoring of therapeutic immune cells both *in vitro* and *in vivo*. Nanogels can also be fabricated to have a core-shell-corona structure in which a shell of inorganic material like silica protects the core of magnetic nanoparticles while its permeability to water allows for the interaction of the water molecules with the magnetic core that is essential for its imaging function. Ruhland et al. reported such nanogels that had a temperature-sensitive corona of crosslinked PNIPAAm. High magnetization and/or bright luminescence was achieved due to a core of maghemite and CdSe(ZnS) while the silica shell provided a strong protection to the core from degradation in conditions of extreme pH, thus giving these nanogels a potential for oral administration [198].

Recently, Wang et al. developed a multifunctional nanogel-based probe for pathological responsive ultrasound and MR imaging [199]. This was achieved by co-loading of two enzymes, catalase (CAT) and superoxide dismutase (SOD) along with SPIO nanoparticles into glycol chitosan-based pH-sensitive nanogels. Most pathological sites have a high concentration of reactive oxygen species, which are converted into molecular oxygen (O_2) by SOD and CAT. O_2 is released in the form of microbubbles that act as a contrast medium for ultrasound imaging. Encapsulation of enzymes within nanogels protects them from inactivation in circulation while the porous and hydrophilic nature of the gel network allows the substrate molecules to diffuse freely within the matrix thus allowing the enzymes to perform their catalytic activity [200,201]. Moreover, the density of cationic charges on the gel surface increases in the acidic pathological microenvironment leading to improved accumulation of nanogels in the target area. In addition, encapsulation of SPIO within the nanogel significantly enhances transverse relaxation rates and generates strong MR signals. The proof-of-concept *in vivo* studies indicated that signals obtained from ultrasound imaging from tumors 1 h after injection are enhanced approximately 7-fold. In the case of MR imaging, significantly darkened tumor signals with approximately 16% enhancement were detected after the injection of nanoprobes.

5. Obstacles to clinical translation and strategies being developed to overcome them

Nanogels, since the time of their discovery, have come a long way and found applications in almost all biomedical fields, ranging from drug therapy to imaging and diagnostics. While a few nanogel-based formulations have reached clinical trials [144-147] for subcutaneous delivery of vaccine antigens, the clinical translation of nanogels for all other diverse applications that they are suitable for remains to be realized. A number of parameters affect the efficacy of this delivery system and requires further optimization. Similar to many other nanomedicines, one of the major drawbacks is that no more than 5-10 % of the injected dose actually reaches the target site, with maximum dose reaching organs involved in clearance like kidney, liver, spleen, etc. [202,203]. The tissue distribution of nanogels is governed by a number of parameters like their size, shape, charge, composition, surface properties and the cargo that they carry. We have attempted to summarize some of the key factors that are

proving to be roadblocks in this journey, along with the alternative approaches being explored to overcome them. However, it must be realized that the final efficacy of the nanogel-based delivery system depends on a complex interplay of all of these factors and an ideal system would be the one with a perfect balance between all of them.

Rapid clearance

Spleen is one of the important organs responsible for the filtration of foreign substances from blood. Splenic filtration is lower for softer particles like nanogels having a size less than 200 nm, that can squeeze through the splenic filtration bed [204,205], while extremely small nanogels (< 20 nm) are usually excreted via renal filtration [70,206], indicating that the size and flexibility of the nanogels have to be well-defined. Shape of the nanogels is also an important parameter that defines their circulation half-life, and filamentous or rod-shaped particles are known to have a considerably longer circulation time than their spherical counterparts [207]. Apart from the size, opsonization or coating by serum proteins also plays a key role in the clearance of the particles. Adsorption of plasma proteins leads to their uptake by the organs of MPS, such as liver and spleen, and can be partially prevented by PEGylation [205,208]. However, PEG is not completely inert, and PEG-specific IgM antibodies are known to be generated after administration of a single dose of PEGylated nanoparticles, resulting in their accelerated blood clearance. This compromises their efficacy on subsequent dosing [209-211]. Furthermore, this response is known to be dependent on the hydrophobicity of the core of carrier to which PEG is conjugated, as well as the type of cargo [209,212]. N-substituted polypeptides or polypeptoids have been recently explored as antifouling agents. Poly (N-methyl glycine) or polysarcosine has the simplest structure in class and possesses a flexible backbone [213,214]. A comparative study of block copolymer micelles, differing only in their hydrophilic block as being either PEG or polysarcosine, revealed considerable overlap between the two systems in terms of their physical properties, solution and assembly behavior, indicating the potential of peptoids like polysarcosine in serving as alternatives to PEG in nanogel systems of the next generation [215].

Charge

The presence of surface charge can alter the opsonization profile of the nanogel, its recognition by cells in the organs of the MPS and its plasma circulation profile. Gel particles having a close to neutral surface charge have been demonstrated to have longer circulation times [216,217]. However, the most important feature of nanogels, their stimuli-responsive behavior, is usually attributable to the charged groups incorporated into network. Moreover, the presence of such groups is often desired to facilitate the binding of drug cargo. Thus it is very difficult to achieve this delicate balance between charge-related responsive behavior and avoidance of the nonspecific interactions of the nanogels with other *in vivo* components. Cationic polymers like PEI are commonly used in the preparation of nanogel carriers for the delivery of nucleic acids. Complexation with negatively charged DNA minimizes the charge-related toxicity of the complexes in circulation; additionally PEG can be conjugated to the nanogels to provide further charge shielding. However, once nucleic acid is released from the carrier intracellularly, the polymer returns back to its native cationic state and is free to show toxicity like shrinkage of the cells, formation of vacuoles and disturbance in the cell cycle [218]. This toxicity was found to be dependent on the molecular weight of PEI used as

well as its dose. To reduce toxicity, polymers based on low molecular weight PEI conjugated via labile linkers were used to prepare the gene carriers [210,219], but it is hard to eliminate the charge effect completely.

Challenges pertaining to biomolecule delivery

The most effective carriers for the delivery of genes are viral vectors; however, due to the limited loading capacity and risk of immune reactions, non-viral delivery systems like those based on nanogels have gained popularity [220,221]. A major limitation encountered by these systems is the low efficiency relative to viral vectors. This is mainly due to the degradation of the nucleic acids in the endosomes and late lysosomes and it is a great challenge to design a delivery system that can facilitate effective endosomal release of the carrier [222,223]. Majority of the synthetic carriers used for the delivery of nucleic acids are cationic in nature in order to form stable complex with the negatively charged nucleic acid to protect them from degradation and to improve circulation time. It has been shown that many of such systems do not remain intact in circulation and are disrupted at the glomerular basement membrane that also carries a negative charge due to the presence of proteoglycans. Thus, the purpose of prolonging the half-life of such agents is defeated [224,225], and the therapeutic agents are cleared by the kidney quite quickly after the disruption of the carrier system [118]. Moreover, the high positive charge density of the carriers increase the propensity of hemolysis and opsonization in circulation, especially if the system is found to be unstable in circulation which can expose the charged residues to serum components. Therapeutic proteins can also be incorporated into nanogels for their delivery. Activity of proteins is highly dependent on their conformational flexibility that makes proteins quite sensitive to various steps involved in formulation development, both physical processes like shear processes experienced during vortexing, and chemical conjugation reactions like crosslinking of the nanogel networks after protein incorporation [226-228]. Careful selection of the process parameters is therefore essential to preserve protein function.

Targeted delivery

In the case of nanogel carriers designed to deliver chemotherapeutics to tumors, the probability of nanogels reaching the core of the tumors, even if they are small enough (30-50 nm) and deformable, is limited which, among other factors, is due to the hampered interstitial transport and the high interstitial fluid pressure [229,230]. In many hypoxic tumors, the vasculature is poorly developed, and almost all large tumors have a tendency to develop a hypoxic and necrotic core, thus making it inaccessible for nanogels via the EPR effect [231]. This approach of passive targeting is therefore useful only for well-vascularized, small tumors or can be potentiated by extravasation/penetration-enhancing pretreatment [77,232,233]. Active targeting of the nanogels by their conjugation to receptor-specific ligands, although can improve the binding of the carrier to specific cells after extravasation, has its own set of challenges. It is often hard to find receptors that are exclusively expressed only on the tissue of interest. For example, folate receptor is overexpressed in a large number of malignancies, but also has a moderate to high level of expression on other normal organs like small intestine, placenta and kidneys [234,235]. Moreover, the expression of receptors on all the cells of the malignant tissue is seldom homogenous, which eventually results in non-uniform accumulation of the delivery system

in that tissue [236-238]. Active targeting approach is also challenging to execute from the perspective of nanogel design. Despite of the recent advances in nanoscale fabrication methods that allow better control over the size and particle size distribution of the nanogels, in majority of cases nanogel samples are heterogeneous, and it is difficult to control the stoichiometry of functional biomolecules on their surface. Biomolecules like antibodies or their fragments, or even peptides are known to be very sensitive to bioconjugation reactions, and can easily lose their binding affinity due to the attachment to nanocarrier [239]. Reproducibility of the nanogel-antibody conjugates in a consistent manner from batch-to-batch is difficult to achieve, since majority of the conjugation reactions use free amines on the lysine side-chain or the cysteine residues obtained from reduction of disulfide bridges in the hinge region of antibodies, which not only results in a variable stoichiometry of conjugation but can also affect the binding affinity of the antibody [240-245]. In recent years, various bioorthogonal chemistries have been successfully developed to achieve site-specific conjugation, but these strategies also required introduction of artificial chemical moieties to the ligand structure and may be less viable for proteins produced through recombinant or bioengineering strategies [246]. In all cases, the introduction of targeting ligands on the surface of the nanogels can also have detrimental effects on their surface characteristics like charge and hydrophobicity, which may lead to increased opsonization, aggregation and clearance by the MPS *in vivo* [247].

Degradation

Because the molecular weight of nanogels is far above the renal threshold (~40 KDa for copolymers), they cannot be removed from the body via the kidneys and if the polymers are nondegradable, so they would tend to accumulate in the body. Even if the nanogels are designed to be degraded into smaller polymer fragments to activate the release of their cargo and eventually facilitate their renal elimination, there is a risk of cellular accumulation of polymer chains by sequestration in the lysosomal compartments. Thus, the use of biodegradable polymers, both natural and synthetic, is most preferable. However, it should be noted that chemical functionalization can alter the pattern and rate of polymer degradation or even render natural polymer nonbiodegradable [248]. Therefore, further investigation of the metabolism and elimination profile of the polymeric nanogels is warranted before they can be proposed for long-term clinical use.

Drug release

Nanogels are designed to be stimuli-responsive for the release of their cargo. At the same time, biodegradable systems are intended to undergo degradation at the target site. Thus, it is difficult to control the rate of both stimuli-responsive drug release and degradation kinetics along with stability in circulation, and it is always a trade-off between these properties that decides the ultimate performance of the delivery system. This may lead to a pattern of release very different from the one usually observed in the *in vitro* experimental settings. Nanogels can also demonstrate burst release for their hydrophilic as well as hydrophobic cargoes which can result in substantial loss of the drug in circulation upon intravenous administration and leaving very little of the drug to be delivered to the target site through the nanogel carrier, while at the same time exposing healthy organs to toxic drugs [249]. These limitations can be addressed partially by altering the composition of the polymers used for

the synthesis of nanogels, but add another layer of complexity to an already intricate system [250,251].

6. Conclusions and future perspectives

As a carrier system, nanogels have evolved over time to be able to encapsulate different types of guest molecules. This is a direct result of the advancement in their synthesis techniques as well as a deeper understanding of their material properties like softness and swelling behavior. This understanding allows us to explore their applications in diverse biomedical fields along with the possibility of fine-tuning these properties to our advantage. Progress in analytical techniques also gives us a better insight about their behavior *in vivo* that can give a direction to efforts being made to improve their pharmacokinetic and degradation profiles to design nanogels of the future.

Nanogels can be designed to be compatible with small molecules like drugs and fluorophores, proteins, peptides, nucleic acids and even inorganic nanoparticles composed of gold, silver, or iron oxide. These tiny carriers can also hold a combination of two or more agents depending on the purpose, and recent years have witnessed the evolution of nanogels as multi-drug carriers and multi-modal imaging agents. They can be surface-functionalized to present targeting ligands to a receptor of interest to home them at the desired site. Nanogels respond to environmental or external stimuli by undergoing volume phase transitions that are manifested in the form of swelling of their crosslinked network. This allows for spatial as well as temporal control of drug release and/or activation of reporter molecules, which in turn can generate signals for the purpose of imaging and diagnosis. These properties allow nanogels to surpass other nanoparticulate systems in terms of their applicability.

Despite the progress made in the field of nanogel design so far, very few nanogels have been explored in the clinical studies. The complexity of the system and intricate structural properties demand careful engineering of the nanogel in order to achieve the desired effect. The scalable production and batch-to batch reproducibility can also be hurdles that need to be addressed. Challenges exist in terms of delivery of the cargo to the desired site as well as efficient clearance of the nanogels once they have accomplished their mission *in vivo*. Although many studies tested the efficacy of nanogel formulations and their safety, reports on their long-term accumulation and degradation profiles are few and far between. Improvements in the design along with detailed investigations regarding the *in vivo* behavior of the nanogels will help in eventually taking them from bench to bedside.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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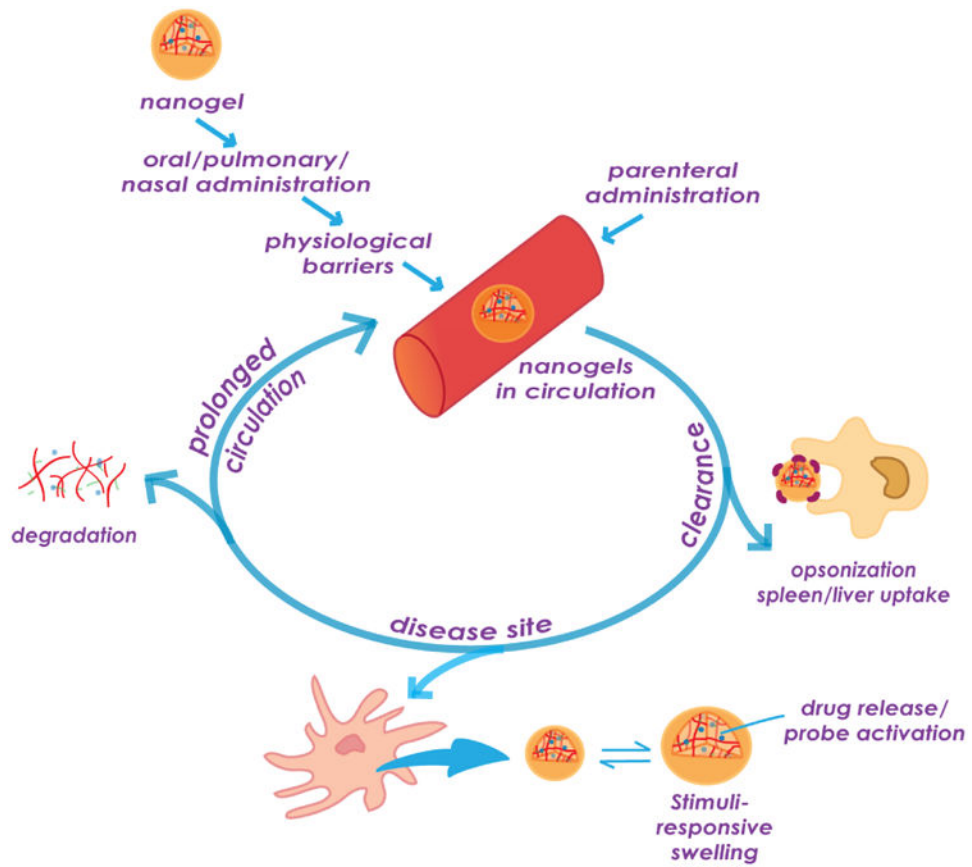
Figure 1.
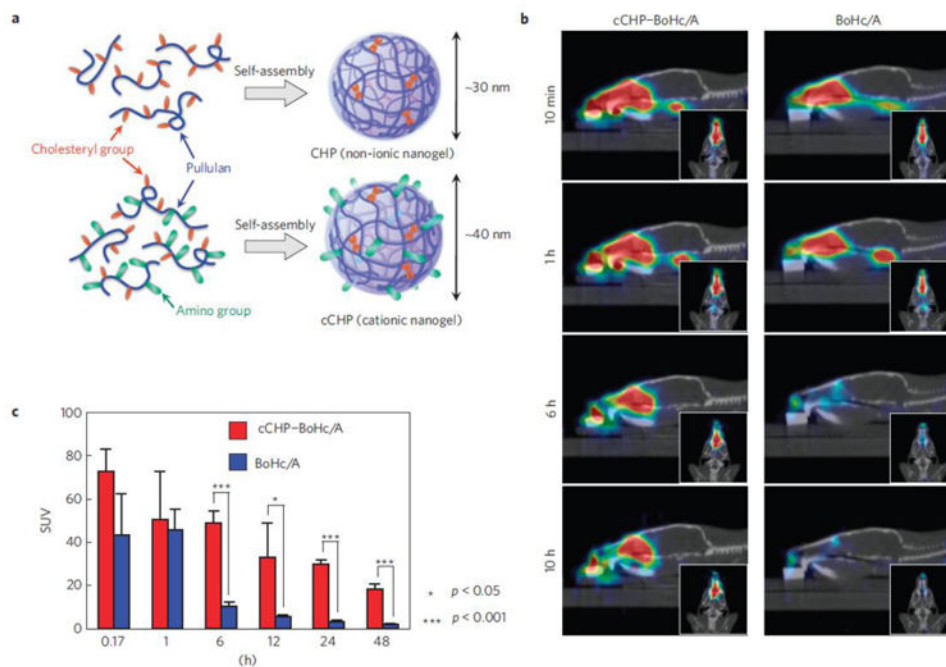*In vivo* behavior of nanogels.

Figure 2.
Use of cCHP nanogel as a new antigen-delivery vehicle for intranasal vaccination. A) Generation of cCHP nanogel from a cationic type of cholesterol-group-bearing pullulan. B) PET images showing that intranasally administered cCHP nanogels carrying [18F]-labelled BoHc/A were effectively delivered to the nasal mucosa. C) Direct quantitative study with [111In]-labelled BoHc/A further demonstrated that BoHc/A was retained in the nasal tissues for more than two days after intranasal immunization with cCHP nanogel. In contrast, most naked BoHc/A disappeared from the nasal cavity within 6 h after administration. Adopted with permission from [14].
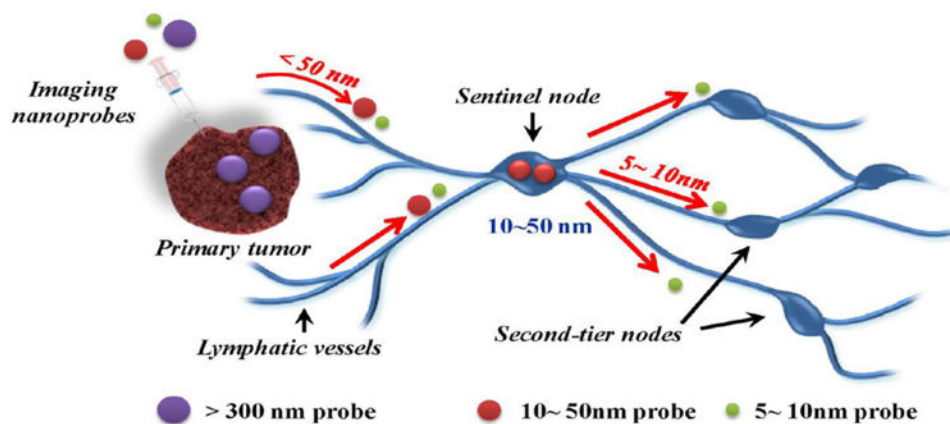
Figure 3.
Schematic illustration of sentinel lymph node (SLN) mapping using a nanoscale imaging probe. Imaging probes on 5 – 10 nm size scale can flow through the SLN into adjacent nodes in the chain; nanoprobes > 300 nm in size rarely leave the injection site, while those with a size range of 10 – 50 nm exhibit rapid uptake into SLN and do not leave. Adapted with permission from [190].
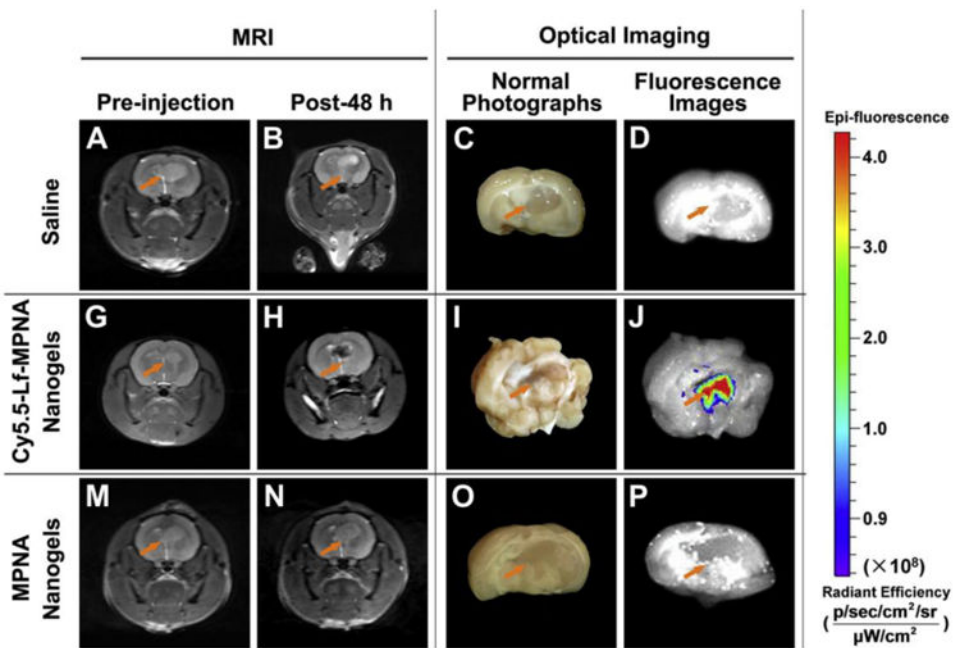
Figure 4.
In vivo studies: representative results of rats bearing gliomas treated with saline as a control (upper row), Cy5.5-Lf-MPNA nanogels (middle row), and MPNA nanogels (lower row), respectively (n = 9 in each group). T_2-weighted MR Images of gliomas before injection (A, G, M) and at 48 h post-injection (B, H, N); normal photographs (C, I, O) and ex vivo fluorescence images (D, J, P) of gliomas at 48 h post-injection; Adapted with permission from [196].