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Advanced nanogel engineering for drug delivery

Koen Raemdonck, Joseph Demeester and Stefaan De Smedt*

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Nanosized hydrogels (nanogels) have attracted considerable attention as multifunctional polymer-based drug delivery systems. Their versatility is demonstrated both in drug encapsulation and drug release. Nanogels can be designed to facilitate the encapsulation of diverse classes of bioactive compounds. With optimization of their molecular composition, size and morphology, nanogels can be tailor-made to sense and respond to environmental changes in order to ensure spatial and stimuli-controlled drug release *in vivo*. This manuscript aims to highlight recent advances in the interface between biology and nanomedicine with the emphasis on nanogels as carriers for controlled drug delivery.

Introduction

In the past decade the quest for intelligent biomaterials in pharmaceutical applications has moved into second gear. More than ever, the design of efficient drug delivery carriers has evolved as a multidisciplinary effort where the interplay between material science, medicine and biology provides innovative biofunctional materials.[1] Amongst the available biomaterials already described in literature, hydrogels have proved their value in diverse biomedical applications. Hydrogels are described as hydrophilic three-dimensional polymer networks that are able to take up large amounts of water or physiological fluid, while maintaining their internal network structure.[2,3] Their high water content and low surface tension contribute to their biocompatibility. Some hydrogels encompass a high loading capacity for bioactive compounds and are able to release their therapeutic payload in a controlled fashion. These unique features explain their widespread application in tissue engineering, drug delivery and diagnostics.[4]

Although research has mainly focused on macroscopic hydrogels, there is now an increasing interest in hydrogels confined to micro- and nanoscopic dimensions (termed micro- and nanogels).[5,6] Colloidally stable nanogel particles have properties similar to those of their macrogel counterparts, but they have the added value that *e.g.* upon intravenous injection, they can be deployed in areas of the body that are not easily accessed by macroscopic hydrogels.[7] As most nanogels are able to be taken up by cells they are ideal candidates for intracellular drug delivery. This is of particular interest for therapeutic agents that need to be safely delivered into the cytoplasm of the target cell, such as antisense oligonucleotides, siRNAs, peptides and various low molecular weight chemotherapeutics. In addition, nanogel dispersions have a large surface area that can function as a template for multivalent conjugations which is important for optimizing the particles towards *in vivo* applications. Moreover, their nanoscaled dimensions ensure that they respond very rapidly to environmental stimuli, which is attractive

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Koen Raemdonck

Koen Raemdonck obtained his Master's degree in Pharmaceutical Sciences from Ghent University in 2004 with great distinction. In the same year he became a doctoral fellow of the Institute for the Promotion of Innovation through Science and Technology in Flanders (IWT-Vlaanderen). He started his PhD in the laboratory of General Biochemistry and Physical Pharmacy under the supervision of Prof. Dr Stefaan De Smedt and Prof. Dr J. Demeester. His doctoral research is focused on the application of nanogels for the controlled intracellular delivery of macromolecules.

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Prof. Dr Joseph Demeester graduated in Pharmaceutical Sciences from Ghent University in 1974 and earned a PhD in Pharmaceutical Sciences in 1980. He became Professor at the same University in 1989 at the Laboratory of General Biochemistry and Physical Pharmacy. Since 1994 he has been the Director of the International Centre for Standards of the International Pharmaceutical Federation (F.I.P.) and since 1998 he has been an expert in the Group on Biological Products of the European Pharmacopoeia. In 2003, Joseph Demeester became President of the Enzyme Commission of the International Pharmaceutical Federation.

when aiming for triggered drug delivery. Hydrogels, and nanogels in particular, hold all the cards to be versatile drug delivery carriers for exploitation in biomedicine, as already discussed in many review articles.[2–6,8,9] In this paper, we especially aim to highlight some of the most recent advances made in this emerging field of nanomaterials, with the emphasis on nanogel design towards efficient *in vivo* application.

Designing nanosized hydrogels

Hydrogel structures can be formed by introducing chemical or physical crosslinks between hydrophilic natural or synthetic polymers.[10] These crosslinks are essential for the structural stability of the hydrogel as they prevent dissolution of the polymer chains in the aqueous environment.

Chemical crosslinking involves the formation of covalent bonds, leading to an insoluble polymer network. To obtain nanogels instead of macro- or microgels by chemical crosslinking, mostly (inverse) emulsion polymerization[11] and precipitation polymerization[12] methods are applied. Alternatively, also physical interactions between polymer chains (*e.g.* hydrogen bonds, chain entanglements, Van der Waals forces, electrostatic interactions…) can result in stable hydrogels. Physical self-assembly implies the controlled formation of nanosized aggregates in the function of polymer concentration[13–15] or environmental parameters such as temperature and pH.[8,16]

We illustrate these different modes of crosslinking taking dextran biopolymer hydrogels as an example.[17] Dextran chains (dex, Fig. 1A) can be derivatized with different moieties such as 2-hydroxyethyl methacrylate (dex-HEMA, Fig. 1B) or allyl isocyanate (dex-AI, Fig. 1C) for radical polymerization to form chemically crosslinked hydrogel particles.[17,18] Alternatively, dextran nanogels were also obtained through respectively stereocomplexation of dextran grafted with poly(*L*-lactide) and poly(*D*-lactide) (Fig. 1D)[15] and inclusion complexation of lauryl-modified dextran (Fig. 1E) with β-cyclodextrin polymers.[19]

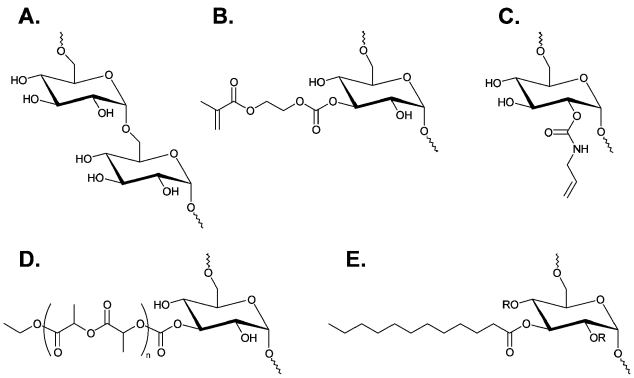

Fig. 1 Chemical structures of various monomers for dextran-based hydrogel particles, (A) dextran, (B) dex-HEMA: dextran hydroxyethyl methacrylate, (C) dex-AI: dextran allyl isocyanate, (D) dex-*g*-PLA: dextran poly(lactic acid), (E) lauryl-modified dextran – R=H or CH_3–$(CH_2)_{10}$–CO–.

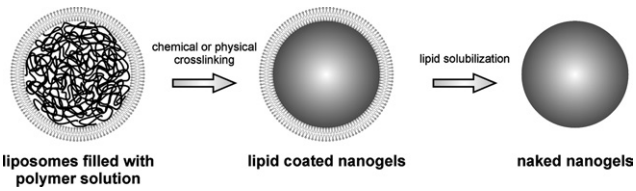

Fig. 2 Synthesis of nanogels using a liposomal template. Liposomes are prepared in the presence of a polymer solution. Non-encapsulated polymer is removed or diluted below the critical polymerization concentration. Crosslinking of the polymers inside the lipid vesicles leads to the formation of lipid coated nanogels. Removal of the lipid coat by *e.g.* the addition of a detergent, finally results in naked nanogels with a size comparable to that of the liposomal template.

Other preparation methods for colloidally stable micro- and nanogels and examples hereof are detailed by Oh *et al.* in a recent review article[6] and by Yallapu *et al.*[8]

Recently, new technologies have arisen for the preparation of nanogels with better defined molecular architectures, which is important to optimize them towards *in vivo* drug delivery applications. One example is the application of liposomes as nanosized reactors where hydrogel formation occurs in the aqueous lumen of the lipid vesicles. In this way, one can achieve better control over the particle size since the dimensions of the 'naked' nanogels, obtained after lipid removal, closely match the size of the liposomal template (Fig. 2).[20–24] Secondly, controlled radical polymerization (CRP) techniques, which offer great 3opportunities for the synthesis of (co)polymers and bioconjugates with controlled molecular weight and low polydispersity,[25] have recently been explored for the synthesis of crosslinked nanogels.[26] Matyjaszewski's group prepared well-defined and uniformly

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crosslinked biodegradable nanogels from oligo(ethylene glycol) monomethyl ether methacrylate (OEOMA) with a disulfide functionalized crosslinker using inverse mini-emulsion atom transfer radical polymerization (ATRP).[27] These nanogels showed potential as drug delivery carriers in their studies.[28,29] Another interesting example is the photolithographic fabrication of sub-micrometre particles using a technique called PRINT (particle replication in nonwetting templates). PRINT applies photocurable perfluoropolyether molds for imprint lithography to form uniform nanoparticles of well-controlled size, shape and composition.[30–32] Gratton and co-workers used PRINT nano- and microgels to investigate the influence of particle size, shape and surface chemistry on their cellular internalization. The experimental data point out that cationic rod-shaped particles are internalized at higher rates, underlining the importance of particle design when intracellular targets are envisioned.[33] An alternative nanoimprint photolithographic approach (step and flash imprint lithography or S-FIL) for the preparation of crosslinked peptide nanogels was recently presented by Glangchai *et al.* (Fig. 3).[34]

Rigorous control over the size of nanocolloids is essential for their *in vivo* biodistribution. While microgels are often designed to release their payload in the extracellular space or in the cytoplasm of dendritic cells following subcutaneous or intramuscular injection, nanogels may also be applied for intravenous administration where they need to extravasate through the capillary endothelium to reach the target tissue. The morphology of the capillary wall will set the size limits for particle extravasation into the surrounding tissue. Discontinuous or sinusoidal capillaries can be found in liver, spleen and bone marrow. This 'leaky' vasculature is also observed in inflammation sites and tumours which allows extravasation of particles even up to 400 nm in diameter.[1,35] Since size is a critical determinant for nanogels to reach the intended target following intravenous administration, new nanofabrication methods with good control over particle dimensions may be promising for *in vivo* applications of drug loaded nanogels. Gao *et al.* recently published on ultrafine polyacrylamide-based hydrogels with an average size ~2 nm for the encapsulation of *meta*-tetra(hydroxyphenyl)-chlorine (mTHPC) as a photosensitizer in photodynamic therapy.[36] The authors state that owing to their small size, these hydrophilic particles will evade the reticulo-endothelial system (RES). Moreover, risk of accumulation of the mTHPC loaded nanogels is also reduced since these non-degradable particles should be cleared from the bloodstream by renal filtration.[36]

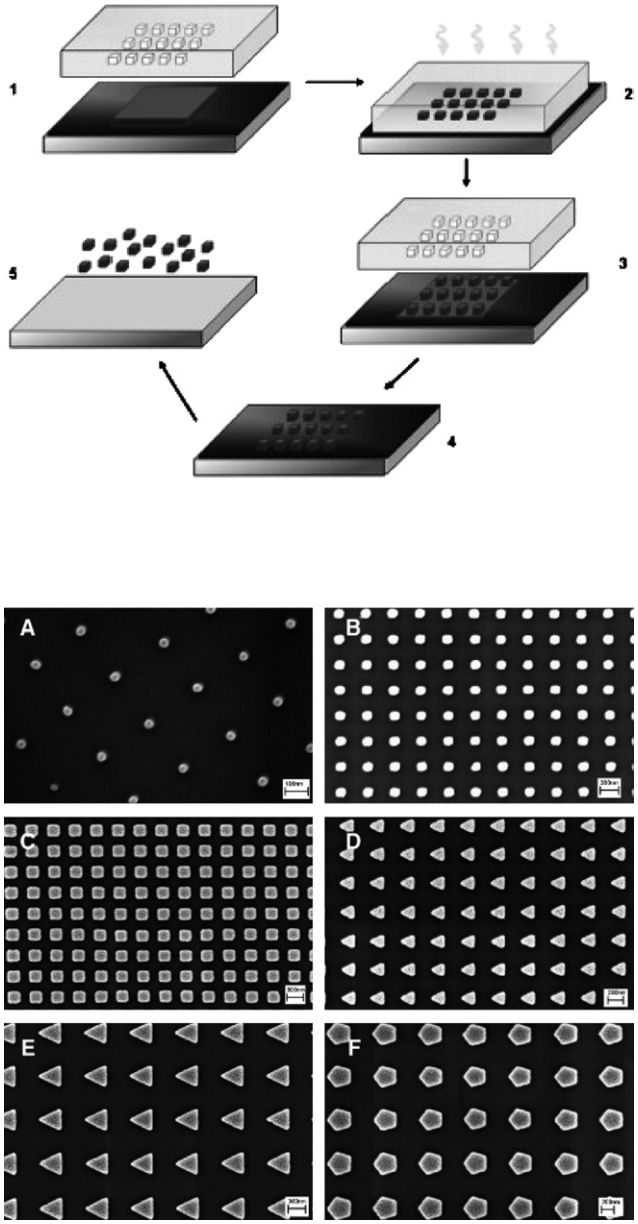

Fig. 3 (Top) Schematic representation of the step and flash imprint lithography (S-FIL) method. Details can be found in Glangchai *et al.*[34] (Bottom) Scanning electron micrographs of S-FIL imprinted poly(ethylene glycol) diacrylate (PEGDA) nanogels. Reprinted with permission from ref. 34. Copyright © 2008 Elsevier Ltd.

Nanogels as versatile drug delivery vehicles

Drug encapsulation

Many drug molecules suffer severely from several impediments such as low solubility, off-target toxicity, instability or inefficient transfer across biological barriers, all of which significantly hamper their *in vivo* use. Nanogels may offer a solution to many of these drug delivery issues, involving various types of

bioactive compounds. Both hydrophilic and lipophilic low molecular weight drugs (*e.g.* certain chemotherapeutics[37]) and macromolecular therapeutics (DNA,[38] siRNA,[39] peptides and proteins[40]) may become incorporated into the nanogel network. The loading is generally based on (a) physical entrapment and/or (b) non-covalent interactions. The steric entrapment of therapeutics inside a hydrogel network implies in most cases that the compounds are present in the polymer solution during the gelation process. However, it is conceivable that polymerization may occur under conditions that are possibly detrimental for the therapeutic cargo. Therefore this encapsulation technique obviously requires rigorous control over the gelation process to ensure drug stability.[34,38,41] When the dimensions of the encapsulated therapeutics exceed the mesh size of the hydrogel network, their diffusional leaching can be prevented. This encapsulation method is therefore most feasible for macromolecular therapeutics,[34,38,39,42,43] especially when loosely crosslinked or porous hydrogels are formed.

In contrast, non-covalent interactions between the biological agent and the polymer matrix are the basis of the research of Akiyoshi and co-workers who focus on amphiphilic cholesterol-modified pullulan (CHP) nanogels of typically 20–50 nm in diameter, mainly for the encapsulation of different proteins.[44] In 1993, Akiyoshi disclosed that polysaccharides partially modified with hydrophobic moieties could form nanoparticles through a self-assembly process in an aqueous environment.[45] The main driving force for protein encapsulation is the hydrophobic attraction between the cholesteryl moieties in the nanogels and hydrophobic nanodomains in the protein of interest. Recently, phase I clinical trials were conducted with CHP nanogels encapsulating truncated HER2 protein for cancer vaccination. Patients received repetitive subcutaneous injections of the CHP-HER2 vaccine and results showed HER2 specific antibody and T-cell immune responses.[46,47] A drawback of this nanogel platform is its limitation for intravenous administration since abundant serum proteins, like albumin, can destabilize the nanogel complex.[44] Especially when an intracellular drug target is envisioned, the stability of drug delivery systems in the extracellular matrix should be routinely tested to avoid premature drug release before the target cells have been reached.[49] For the intracellular delivery of membrane-impermeable compounds, amino-functionalized CHP nanogels were proposed to improve the efficiency of interactions with the cellular membrane and their subsequent internalization. Once the cellular barrier is overcome, the authors assume that the aforementioned protein exchange mechanism is also partially responsible for the intracellular release of the encapsulated therapeutic protein.[40]

An alternative non-covalent approach for the incorporation of therapeutics in charged nanogels is based on electrostatic interactions between the biological agent and the ionized polymer matrix. This approach has proved valuable for *e.g.* the complexation of low molecular weight anionic compounds like nucleoside analog 5′-triphosphates (NTPs) in crosslinked poly(ethylene glycol)-poly(ethylene imine) (PEG-*g*-PEI) nanogels (Fig. 4).[48] Electrostatic complexation can also be exploited for encapsulation of polynucleotides in cationic nanogels if the pore size of the nanogels is large enough to allow penetration of the polynucleotides into the interior of the gel particle. In this way, antisense oligonucleotides[50–52] and short interfering RNA (siRNA)[18,52] can be loaded into preformed cationic hydrogel networks.

Exceptionally, bioactive compounds are incorporated into the nanogel interior through covalent conjugation. One example is given by Standley and co-workers who linked a methacrylate-modified CpG oligonucleotide ligand into acid-degradable nanogels prepared through an inverse emulsion radical polymerization technique.[43] In addition to the oligonucleotide strand, also ovalbumine (OVA) as an antigen model was encapsulated. The incorporation of CpG oligonucleotides resulted in a more potent production of interleukin-12 and enhanced OVA-specific CD8 T-cell immunity *in vivo* due to activation of Toll-like receptor 9 (TLR-9). However, covalent attachment is not feasible for every type of drug or application as it may also alter the drugs' effectiveness.

Triggered drug release

Hydrogels tend to swell when they are brought into a polymer compatible medium due to solvent penetration into free spaces between the macromolecular chains. The resulting volumetric expansion of the hydrogel network is governed by the balance between the internal osmotic pressure and its elastic deformability.[53] An attractive feature of hydrogels is that their swelling behaviour can be influenced by a diverse range of external triggers, such as changes in environmental pH, ionic strength or temperature and the application of light or a magnetic or electric field.[3,5,54] A plethora of stimuli-responsive nanocarriers is described in literature, *e.g.* polymer-drug conjugates, liposomes and micelles, which all have their strengths and weaknesses.[55] The latter two examples are of particular interest in drug delivery, but they suffer

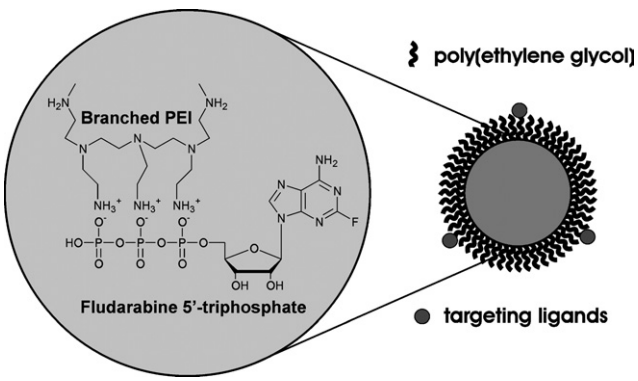

Fig. 4 Schematic representation of PEG-g-PEI nanogels, electrostatically loaded with 5′-nucleoside triphosphate anticancer agents (5′-NTPs). The nanogels consist of a PEI core for 5′-NTP binding and a PEG envelope, containing targeting ligands (●). Reprinted and modified with permission from Vinogradov *et al.*[48] Copyright © 2005 Elsevier Ltd.

from drawbacks such as a lack of controlled loadability, limitations in material composition, limitations in the type of biological cargo that can be loaded/released and/or physical instability.[7,8,30] For these reasons, many nanogel platforms are often regarded as a better alternative. The responsiveness of 'intelligent' hydrogels is largely dependent on the type of polymer used in making the gel.[56,57] Control over the swelling-deswelling transitions or other physical changes on a nanoscopic scale are especially of interest for intracellular controlled drug delivery.

Among the class of 'intelligent nanogels', those responding on changes in temperature and pH are most extensively investigated. Nanogels constructed from polymers bearing pendant basic or acidic groups (ionic nanogels) are generally regarded as pH responsive.[57] When they are dispersed in an aqueous medium of appropriate pH and ionic strength, ionization of pendant groups occurs, thereby introducing fixed charges in the polymer network. By electrostatic repulsion the pores in the nanogels enlarge, allowing excessive solvent influx, which eventually leads to their swelling. Cationic nanogels swell at a pH below the pK_a of the polymer network, while anionic nanogels are ionized at higher pH. Small changes in the external pH may have a significant influence on the swelling behaviour.[3] Na and Bae reported on self-assembled pH-sensitive nanogels, composed of a sulfonamide-pullulan acetate conjugate[58] and hydrophobized pullulan-Na-Boc-L-histidine[59] for the tumour specific release of doxorubicin. Most solid tumours elicit a lower extracellular pH (pH $\approx$ 6.8) when compared with the pH in other tissues or blood circulation (pH 7.4).[55,60,61] The particles proposed by Na and Bae were stable at pH 7.4. However, at pH 6.8 the sulfonamide (SA) tagged nanogels shrank and aggregated due to SA deionization while the histidine tagged nanogels swelled as a result of imidazol protonation in the histidine moieties. In both cases drug release occurred faster in response to these physical changes resulting into a more specific deposition of the drug in the tumour microenvironment.

In the intracellular microenvironment, such pH responsive carriers may also prove beneficial. It is known that nanocarriers are internalized mostly through an endocytotic mechanism upon interaction with the cellular membrane. Endocytosis eventually confines the particles in an acidic environment as the pH in the late endosomal and lysosomal lumen gradually lowers from neutral to $\sim$pH 5.[62] Indeed, pH-sensitive nanogels that undergo structural changes upon endocytosis could enhance the intracellular bioavailability of the encapsulated drug. Chitosan nanogels around 180 nm in diameter where described by Zhang and colleagues for the encapsulation of the anticancer agent metotrexate disodium (MTX).[63] The nanogels originated from the ionic crosslinking of N-[(2-hydroxy-3-trimethylammonium)propyl] chitosan chloride (HTCC) with sodium tripolyphosphate (TPP) counterions. MTX is negatively charged and could be incorporated in the cationic HTCC nanogels at physiological pH through electrostatic binding. Following efficient cellular internalization, these nanogels showed a 2.2 fold increase in hydrodynamic diameter at endolysosomal pH due to higher protonation of primary and secondary amines in HTCC and stronger electrostatic repulsion. As a result, a faster diffusion driven release of MTX was achieved.[63] Shen *et al.* prepared chitosan nanogels $\sim$70–80 nm through the crosslinking of chitosan with ethylenediamine tetraacetic acid (EDTA). These nanogels exhibit a reversible change in surface charge and surface structure depending on the environmental pH. The authors attempted to exploit this behaviour for the encapsulation and release of the water-insoluble anticancer drug camptothecin.[64] Similarly, Nagasaki's lab showed pH-triggered release of doxorubicin due to protonation and subsequent swelling of ionic PEG-poly[2-(*N,N*-(diethylamino)ethyl methacrylate] nanogels at endolysosomal pH.[65,66] In particular for biomacromolecules like protein antigens, pDNA and siRNA, an efficient transfer from the endosomal compartment into the cytosol is crucial since otherwise they become degraded by endolysosomal proteases and nucleases. Employing pH-sensitive nanogels could enhance this endosomal escape significantly. Indeed, it is thought that when nanogels are constructed from polyelectrolytes with endosomal buffering capacity, they can disrupt endosomes *via* an osmotic pressure buildup (proton sponge hypothesis). In addition, the swelling of the particles, due to endosomal protonation, may facilitate endosomal destabilization.[67,68]

The pharmaceutical relevance of thermosensitive nanogels lies in the treatment of local infections (with elevated environmental temperature), tumour hyperthermia or by artificially increasing temperature in the affected tissue to tailor local drug delivery.[7,55] Accumulation of a drug loaded nanoparticle at the disease site, followed by a local temperature increase may trigger drug release. Poly(N-isopropylacrylamide) or PNIPAAm is the most widely investigated thermoresponsive polymer for biomedical applications.[56] PNIPAAm nanogels can exhibit significant volume transitions in response to thermal fluctuations.[69–72] In aqueous solution the polymer has a lower critical solution temperature (LCST) around 32 °C. Below the LCST, the polymer network is hydrated by hydrogen bonding of water to the amide side groups. When the temperature increases towards or above the LCST, the polymer-polymer hydrophobic interactions become more dominant. As the hydrogen bonds are weakened, phase separation occurs and the polymer chains collapse. The resulting expulsion of water and nanogel aggregation can be accompanied with the release of both hydrophilic and hydrophobic drugs in the surrounding medium.[73] Modification of NIPAAm with different comonomers or post-polymerization modifications on PNIPAAm nanogels may significantly alter the hydrogel characteristics and shift the LCST. This feature enables the application of PNIPAAm nanogels over a wide temperature range.[71] As an example, incorporation of ionic and hydrophilic comonomers, such as acrylic acid (AAc) or methacrylic acid (MAAc), increases the LCST and makes the nanogels sensitive both to changes in temperature as well as changes in pH.[68,75–80] A plethora of reports, describing different modifications of NIPAAm, can be found in literature.[5,6,56,79–81] A particularly interesting example of nanogel engineering is the development of amphoteric, glucose-responsive PNIPAAm based nanogels for triggered insulin release.[82] Grafting of phenylboronic acid (PBA) to cationic-anionic nanogels introduces glucose-responsiveness into the nanogels. The

swelling-deswelling behaviour of the nanogels as a function of physiological glucose concentrations can be fine-tuned by varying the relative amount of PBA, cationic and anionic groups in the PNIPAAm hydrogel particles.[82] Besides pH and temperature sensitive nanogels, also nanogels which are sensitive simultaneously to multiple triggers have already been prepared. For instance, Bhattacharya *et al.* engineered hybrid nanogels that contain ferromagnetic nanoparticles and which are responsive to both temperature, pH and magnetic fields.[83]

Depending on the elastic properties of the polymer network, the deformation of hydrogels can be reversible. In this way, hydrogels can regain their original shape and structure when the stimulus, triggering the swelling or shrinking, is removed. This 'shape memory effect' can also have its implications on drug delivery as recently disclosed by You Han Bae's group. They prepared nanogels consisting of a pH-sensitive hydrophobic core of poly(*L*-histidine-*co*-phenylalanine), held together by a double hydrophilic shell.[74] These nanogels are colloidally stable at neutral pH, but swell substantially at endosomal pH (<pH 6.8) explaining the faster release rate of encapsulated doxorubicin at lower pH values. After endocytosis, the swelling of the nanogels together with the buffering effect of poly(*L*-histidine) is considered to disrupt the endosomal membranes, thereby facilitating the transfer of the nanogels and the released doxorubicin to the cytoplasm. There the nanogels shrink again to their original size, imparting further release of doxorubicin. The doxorubicin released intracellularly induces apoptosis that allows the nanogels, still containing sufficient amounts of doxorubicin, to be taken up by neighbouring cells. In this way, doxorubicin loaded nanogels may 'infect' several cells and release the anticancer agent in a pH-triggered pulsatile fashion (Fig. 5).[74]

Different types of degradable nanogels are currently explored with the aim of enhancing the intracellular delivery of encapsulated drugs. Degradability of the nanogel carrier is essential in drug delivery to lower toxicity and avoid accumulation in the body upon repeated administration. Labile bonds can be incorporated in the polymer backbone or in the crosslinks of the hydrogel network.

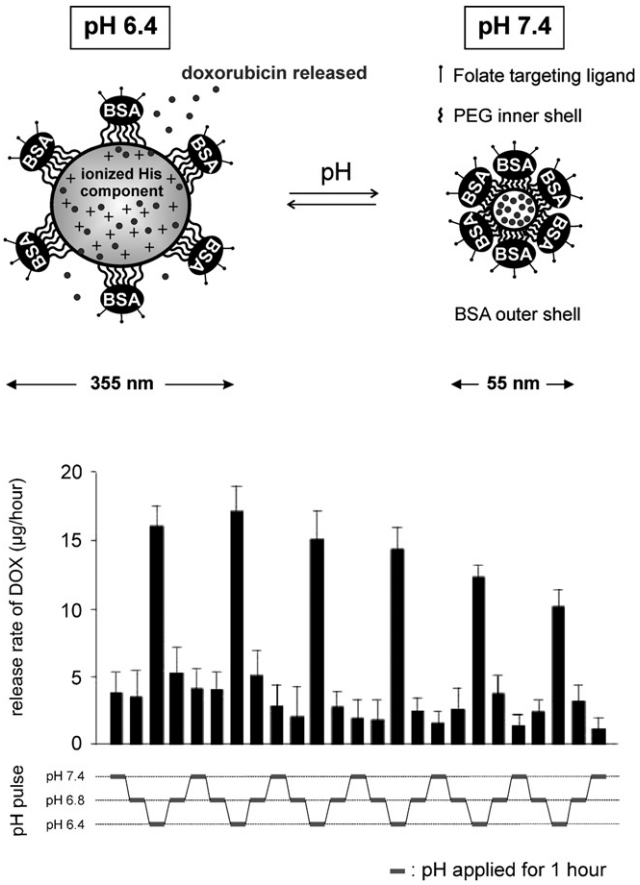

Fig. 5 (Top) Protonation of histidine moieties in poly(*L*-histidine-*co*-phenylalanine) nanogels at lower pH results in significant swelling of the nanogel core triggering doxorubicin (DOX) release. (Bottom) Pulsatile release pattern of doxorubicin from poly(*L*-histidine-*co*-phenylalanine) nanogels as a result of swelling/deswelling transitions in response to a change in environmental pH. Graph reproduced with permission from Lee *et al.*[74] Copyright © 2008 Wiley-VCH Verlag GmbH & Co. KGaA.

This class of nanogels releases its payload upon degradation in response to specific intracellular stimuli such as pH,[41] reducing agents or enzymatic activity. As a first example, the Fréchet group reported on DNA and protein antigen encapsulation into acrylamide nanogels copolymerized with an acid cleavable bisacrylamide acetal crosslinker.[38,42,43] These nanogels are designed to degrade under the acidic conditions found in the phagolysosomes of antigen presenting cell's (APCs). Upon lysosomal degradation of the nanogels, the encapsulated DNA is released and interacts with TLR-9 for enhanced cytokine production.[38,43] Moreover, the accumulation of degradation fragments in the lysosomal lumen induces a rise in osmotic pressure which is believed to destabilize the endosomal membrane and enables the transfer of the encapsulated antigen to the cytoplasm for MHC class I processing.[42,43] Second, disulfide crosslinked nanogels employ the intracellular reductive potential to deliver their therapeutic payload.[84] It is known that disulfide linkages can be selectively cleaved inside the target cell by the reductive environment in the endo/lysosomes and by cytoplasmic glutathione.[85] Lee *et al.* succeeded in the fabrication of hyaluronic acid (HA) based nanogels around 200–500 nm in size.[39] For this purpose, thiol modified HA was crosslinked by ultrasonic treatment through the formation of disulfide bonds in an inverse water-in-oil emulsification process. SiRNA was added to the HA solution during the emulsification process which led to their physical entrapment inside the resulting nanogels. Upon cellular internalization, disulfide reduction disassembled the nanogels which resulted in siRNA release.[39] In

a comparable approach, cationic PEI-*cl*-PEG/Pluronic nanogels[86] were made degradable by the use of branched PEI polycations in which the 2 kDa PEI segments were linked *via* disulfide bridges.[85] Very recently, Morimoto *et al.* reported on the self-assembly of PNIPAAm-*g*-pullulan chains carrying thiol end groups, resulting in nanogels responding to changes in environmental temperature and redox potential. Below the LCST, only disulfide bridges secure nanogel stability, while above the LCST the hydrophobic interactions between the PNIPAAm side chains introduce additional physical crosslinks in the hydrogel network.[87] Third, intracellular release can also be triggered by enzymatic degradation of the nanogels. Mostly macro- and microscopic enzyme-responsive hydrogels have been investigated so far.[88–90] However, Glangchai *et al.* recently demonstrated the lithographic preparation of enzymatically-triggered nanogels.[34] The authors designed sub 100 nm sized PEG diacrylate nanogels that are copolymerized with an acrylated GFLGK peptide crosslinker. Enzymatic cleavage of the crosslinker by thiol proteases, *e.g.* cathepsin B, releases the therapeutic payload which was shown for antibodies and pDNA.[34] As this type of enzyme is mainly located in the extracellular matrix of tumours and inside lysosomes, the drug will be mainly released locally in these confined compartments.

Optimizing nanogel architecture for in vivo application

Ideally, drug delivery vehicles have to safely guide their therapeutic payload to the desired target tissue. However, to reach the target tissue, several extracellular barriers need to be overcome.[1] Shielding of the nanoparticle surface with inert, hydrophilic polymers and introducing targeting ligands may significantly improve *in vivo* delivery and therapeutic efficacy (Fig. 6). The hydrophilic modification of nanoparticles can (a) prevent particle uptake by the mononuclear phagocytic system, (b) decrease the recognition by the immune system and (c) enhance their circulation time in the bloodstream. Indeed, the presence of a hydrophilic surface is known to reduce non-specific interactions with serum proteins and thereby also lowers the risk of phagocytosis by immune cells as opsonisation is prevented.[91] Longer circulation times offer nanogels a better chance of targeting the site of interest and may increase passive accumulation of drug loaded nanogels into distant tumours due to the enhanced permeability and retention (EPR) effect. It has been described that the leaky discontinuous vasculature often found in tumour tissue, together with the slower efflux, can result in high local deposition of nanoparticles.[55,92,93] In addition, targeting of cell-surface receptors is a very attractive concept to achieve cell specific binding and subsequent internalization. In this way, the fraction of the administered dose that accumulates at the target site can be enhanced thereby also minimizing the risk on adverse effects. Many recent investigations have evaluated both strategies for drug loaded nanogels.

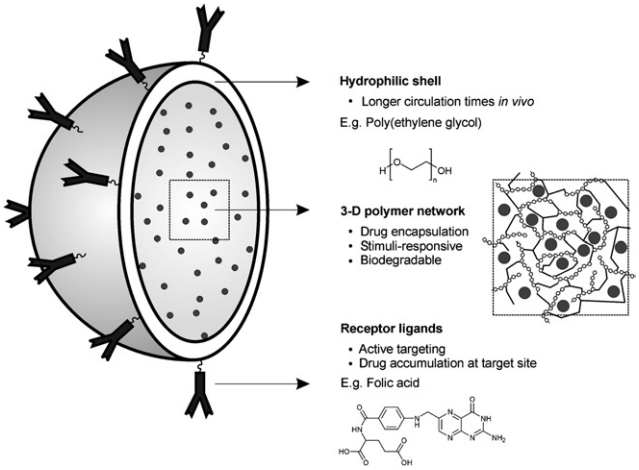

Fig. 6 Biofunctionalization of stimuli-responsive nanogels through the introduction of a hydrophilic shell and the coupling of targeting ligands may result in nanogel carriers that are of interest for clinical applications.

With regard to the shielding of nanogels, most research is focused on PEG based nanogels or the surface modification of nanogels with a hydrophilic PEG shell (also termed PEGylation). Several groups constructed stimuli-responsive core-crosslinked polymeric micelles with a PEG corona.[94,95] However, some controversy exists on whether or not this type of nanocarrier can be regarded as a nanogel. Nagasaki and co-workers copolymerized PEG, carrying a vinylbenzyl group, with 2-(*N,N*-diethylamino)ethyl methacrylate to obtain cationic nanogels with a hydrophilic PEG shell.[65,66,96] Lee *et al.* fabricated a double hydrophilic shell, consisting of PEG and bovine serum albumin (BSA), at the surface of polypeptide nanogels.[74] Besides PEG, other hydrophilic polymers have also been investigated for their 'stealth' properties, such as various polysaccharides.[97–99] With regard to the active targeting of nanoparticles, the iron transporting plasma protein transferrin is frequently used as a targeting ligand for tumour targeting since the expression of the transferrin receptor is often upregulated in fast-dividing tissues.[100] Chitosan,[63] poly(NIPAAm) based[76,81] and PEG-*cl*-PEI nanogels[86] were modified with transferrin to improve their cellular uptake and intracellular drug delivery. The folate receptor is also overexpressed in many tumours,[101] making folic acid a prime candidate for active targeting of nanogels.[48,68,69,74,102] Other valuable approaches include incorporating ligands for the asialoglycoprotein receptor, used in hepatocyte targeting,[77,103] or the conjugation of monoclonal antibodies for specific cell-surface markers.[52,104]

Conclusions and future perspectives

Owing to our increasing knowledge on the biological barriers that nanomaterials encounter *in vivo*, research in the field of drug delivery has gradually evolved to a top-to-bottom approach.[1] This approach implies that scientists now aim to design nanoparticles with appropriate drug delivery properties in relation to the envisioned biological target. Nanogels fit extremely well to this strategy, since they

offer the possibility for stimuli-controlled release of therapeutic agents. Several proof-of-concept studies have highlighted the potential of such 'intelligent' nanogels that are able to sense and respond to environmental changes. In this way, drug release can be controlled in a spatial and temporal fashion. Especially attractive so far has been the design of nanogels that retain their therapeutic payload in the extracellular environment but release it once they are internalized by target cells, in response to an intracellular trigger. However, like for all types of nanoparticles under investigation for drug delivery, the *in vivo* biodistribution of nanogels is mainly governed by their size and surface properties which should also be taken into careful consideration in the engineering of nanogels for clinical applications.

Despite the progress made in this field of nanomaterials there still is a long way to go before drug loaded nanogels will reach the clinic. More than a decade of intensive research provided the technological basis for the development of 'intelligent' soft nanomaterials. Nonetheless, new developments both in engineering stimuli-responsive materials, and bioconjugation methods as in controlled colloid synthesis are key considerations to further improve the design of advanced hydrogel nanomaterials. Moreover, it must be emphasized that there is an urgent need for *in vivo* data from nanogels in preclinical development. To translate the nanogel concept into a viable therapeutic approach, it will indeed become increasingly important to study toxicity, immunogenicity and pharmacokinetics together with drug effects in validated *in vivo* models.

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