REVIEW

Polymeric Nanogels as Drug Delivery Systems

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Summary

The present review focuses on the description of the design, synthesis and physico-chemical and biological evaluation of polymer nanogels. Nanogels are robust swollen cross-linked polymer nanoparticles that can be used as highly efficient and biodegradable carriers for the transport of drugs in controlled drug delivery. In this article, various types of nanogels are described and methods for their preparation discussed. The possibility of using synthesized nanosystems for targeting are reviewed to show the potential of tailored structures to reach either solid tumor tissue or direct tumor cells. Finally, the methods for encapsulation or attachment of biologically active molecules, e.g. drugs, proteins, are described and compared.

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Introduction

By definition, nanogels are three-dimensional networks formed by cross-linked polymer chains. Since they are made up of hydrogel particles of nanometers in size, hydrogel and nanoparticle properties occur simultaneously in nanogels. They can be successfully prepared from polymeric precursors or prepared by heterogeneous polymerization of monomers. A key step in the preparation of nanogels is physical or chemical cross-linking (Bae *et al.* 2005, Oh *et al.* 2007, Hamidi *et al.* 2008).

Moreover, nanogel-based nanomedicines should fulfill all the requirements for drug delivery systems. To ensure maximum therapeutic effect with minimal side effects, stable covalent bonds or, less preferably, encapsulation of the active substance must be guaranteed. Indeed, premature release during circulation and in healthy tissues should be reduced and subsequent release at the desired site ensured. An additional important requirement is the possibility of both passive and active targeting to the place of the action. Passive targeting is very important in the targeted treatment of localized vascularized solid tumors or localized points of inflammation. Naturally, it is important for nanogel-based drug delivery systems to be completely non-toxic, non-immunogenic and biocompatible and to be biodegradable to non-toxic degradation products that can be eliminated from the body (Chacko *et al.* 2012).

Properties of nanogels

Nanogels are nanoparticles with a particle size of 100-200 nm composed of a hydrogel nanoparticles (Fig. 1) with a particle formed by cross-linking a hydrophilic polymer (Garg *et al.* 2012). The small size of nanogels enables their rapid response to environmental changes, such as change in pH or temperature (Soni and Yadav 2016). They can be formed by physically or chemically cross-linked synthetic polymers (Bencherif *et al.* 2009) or biopolymers (Kabanov and Vinogradov 2009). Due to their large inner surface area, nanogels have a very

high loading capacity for carried active molecules (Soni and Yadav 2016). This enables the active transport of drugs with significantly prolonged circulation time and increase in the stability in the biological environment (Bae *et al.* 2008, Soni and Yadav 2016). Generally, nanogels are used to transport biologically active molecules, low- or high-molecular weight active molecules or biomacromolecules (Lee *et al.* 2007, Bae *et al.* 2008). In particular, the controlled delivery and release of a wide variety of low-molecular weight drugs has been discussed in the literature (Sharma *et al.* 2016). Nanogels occur in the form of a three-dimensional structure in which drugs, bioactive copolymers and dispersed liquid phases can be captured (Vintiloiu and Leroux 2008). Furthermore, polymeric nanogels can be chemically modified to contain various ligands for actively targeted drug delivery with possible drug release options (Vinogradov *et al.* 2005). Interestingly, minimally cross-linked polyelectrolyte nanogels may incorporate biomacromolecules with the opposite charge (Kabanov and Vinogradov 2009).
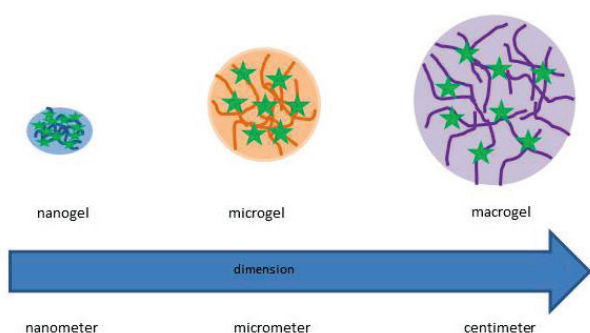

Fig. 1. Schematic representation of polymeric gels of various dimensions.

Generally, nanogels have the following advantages from the biomedical application point of view (Hayashi *et al.* 2004, Hamidi *et al.* 2008, Shidhaye *et al.* 2008, Kabanov and Vinogradov 2009, Uthaman *et al.* 2014, Sivaram *et al.* 2015, Sharma *et al.* 2016):

- Biocompatibility and biodegradability under physiological conditions, without causing an immune response
- Stability and dispersibility in aqueous solutions
- Enhanced bloodstream transport and tissue permeation properties due to their optimal nm scale size
- Response to a wide range of external stimuli (ionic strength, pH, temperature)
- Characteristics suitable for controlled delivery of hydrophilic and hydrophobic drugs
- Integration of active nanoparticles (NPs), e.g. magnetic NPs, in the range of few nm within their network
- The density of the cross-linking can regulate drug release and also greatly affect the size of the prepared nanogels
- High capacity for absorbing large amounts of water or biological fluids
- Versatile construction with flexible size and large surface
- The controlled release of a wide range of drugs
- The ability to protect their cargos against degradation *in vivo*
- Prolonged circulation time of carried drugs
- The availability of various polymer materials suitable for nanogel formation and thus the change of nanogel property.

The general classification of nanogels is based on their structure motifs. Individual types of nanogels and their examples are listed in Table 1.

Synthesis of nanogels

Universally, nanogels can be synthesized using four different procedures, schematically depicted on Figure 2 and listed below.

Physical self-assembling of interacting polymer chains

Physical interactions between polymers can be divided into electrostatic interactions, Van der Waals interactions, hydrogen bonds and hydrophobic interactions (Zamurovic *et al.* 2007). Physical self-assembly in the case of nanogel formation usually involves controlled aggregation of amphiphilic or hydrophilic polymers capable of interacting *via* hydrophobic or electrostatic interactions and/or hydrogen bonds. Usually, the preparation of these nanogels is carried out under mild conditions in an aqueous medium. The size of nanogels is controlled by appropriate selection of polymer concentration, amphiphilic character, functional groups, pH, ionic strength and temperature.

For example, polysaccharide-based nanogels can be prepared by this way. Polysaccharides are used as hydrophilic polymers which are modified by hydrophobic groups. In the case of such modified polymers, the hydrophobic moieties interact with each other and thus the formation of nanoparticles suitable for the transport of active substances is significantly

increased (Oh *et al.* 2009).

Another example of hydrophobic interaction-based nanogel formation are systems based on cholesterol-modified pullulans. These systems can be used for transporting various bioactive molecules, for example insulin (Akiyoshi *et al.* 1998). Hydrophobic interaction can occur even between two types of polymers. For example, lauryl-modified dextrans with β-cyclodextrins containing polymers were used for successful nanogel formation (Daoud-Mahammed *et al.* 2007).

The non-covalent interactions between the polymer chains are relatively weaker interactions when compared to covalent bonds. Therefore, stable nanogels of manageable size may be less easily prepared using this approach. On the other hand, physically cross-linked nanogels have a number of advantages over chemically cross-linked nanogels, as no cross-linker and/or catalyst, which may be toxic, is required. However, their lower stability in biological media often does not enable their use in biomedical applications (Uthaman *et al.* 2014).

Table 1. Nanogel structure classification.

Type of nanogel	Network structure	Example
Simple nanogel	Cross-linked by self-aggregation	Cholesterol-bearing pullulan nanogel (Inomoto *et al.* 2009, Fujioka-Kobayashi *et al.* 2012, Morimoto *et al.* 2013, Bal *et al.* 2018) or cholesterol-bearing enzymatically synthetized amphiphilic glycogen (Takahashi *et al.* 2011)
	pH and temperature responsive semi-interpenetrating polymer network	Hydroxypropylcellulose-poly(acrylic acid) (HPC-PAA) with hydroxyl group of HPC modified by CdSe (Wu *et al.* 2010, Nan *et al.* 2014)
Hollow nanogel	Interpenetrating polymer network	Poly(acrylicacid) (PAA) network and poly(N-isopropylacrylamid) (PNIPAM) network (PNIPAM/PAA IPN hollow nanogels) (Xing *et al.* 2011)
Core-shell nanogels	Magnetic particles encapsulated by the synthetized polymer gel	Poly(acrylamide) or poly(acrylamide-vinyl amine) (shell) with magnetic core (Fe_3O_4 nanoparticles) (Sun *et al.* 2006, Li *et al.* 2017)
Hairy nanogel	Cross-linked by RAFT aqueous dispersion polymerization	Core-shell nanogel containing linear poly(ethylene glycol) and/or nonlinear polymer with oligo(ethylene glycol) side chains (Shen *et al.* 2011, Fu *et al.* 2017)
Multilayer nanogels	Cross-linked by dispersion polymerization	Poly(NIPAM-co-AA-co-rhodamine) nanogel with layer-by-layer assembly of polyelectrolytes (Wong *et al.* 2009)
Functionalized nanogels	Cross-linked by three-step cross-linking	Diblock copolymer of poly(ethylene oxide)-b-poly(methacrylic acid) (PEG-b-PMA) with folate targeting group (Nukolova *et al.* 2011, Lv *et al.* 2018)

Polymerization of monomers in a homogeneous phase or a micro- and/or nano-heterogeneous phase

Polymerization suitable for nanogel formation can be divided into two types, emulsion and inverse emulsion polymerization. In the latter, inverse water in oil nano-emulsion as a medium for the polymerization of monomers is used. Indeed, stable nanogels are obtained

after the addition of specific co-monomers, which serve as bifunctional cross-linkers. The Khmelnitsky group (Khmelnitsky *et al.* 1992) described covalently immobilized enzymes in polymer nanogels based on the copolymerization of acrylamide with *N,N*-methylene-*bis*-acrylamides. Polymerization leading to nanogels may also be carried out in oil in water nano-emulsion or in an

aqueous suspension. In some cases, the polymerization can be initiated in a homogeneous aqueous solution, which changes during polymerization to a milky suspension containing the growing nanogel. The final product is then separated from suspension by freeze-drying. One example is the synthesis of poly(methacrylic acid-grafted-poly(ethylene glycol)) nanogels, which are promising candidates for oral protein delivery, in water

by using a combination of UV-initiated free-radical solution/precipitation polymerization. Methacrylic acid, O-(methacryl)-O'-methylpolyethylene glycol with different molecular weights (200, 400 and 1,000) were used as monomers, tetraethylene glycol dimethacrylate was used as a cross-linker and 1-hydroxycyclohexyl-phenyl ketone was used as UV initiator (Donini *et al.* 2002).
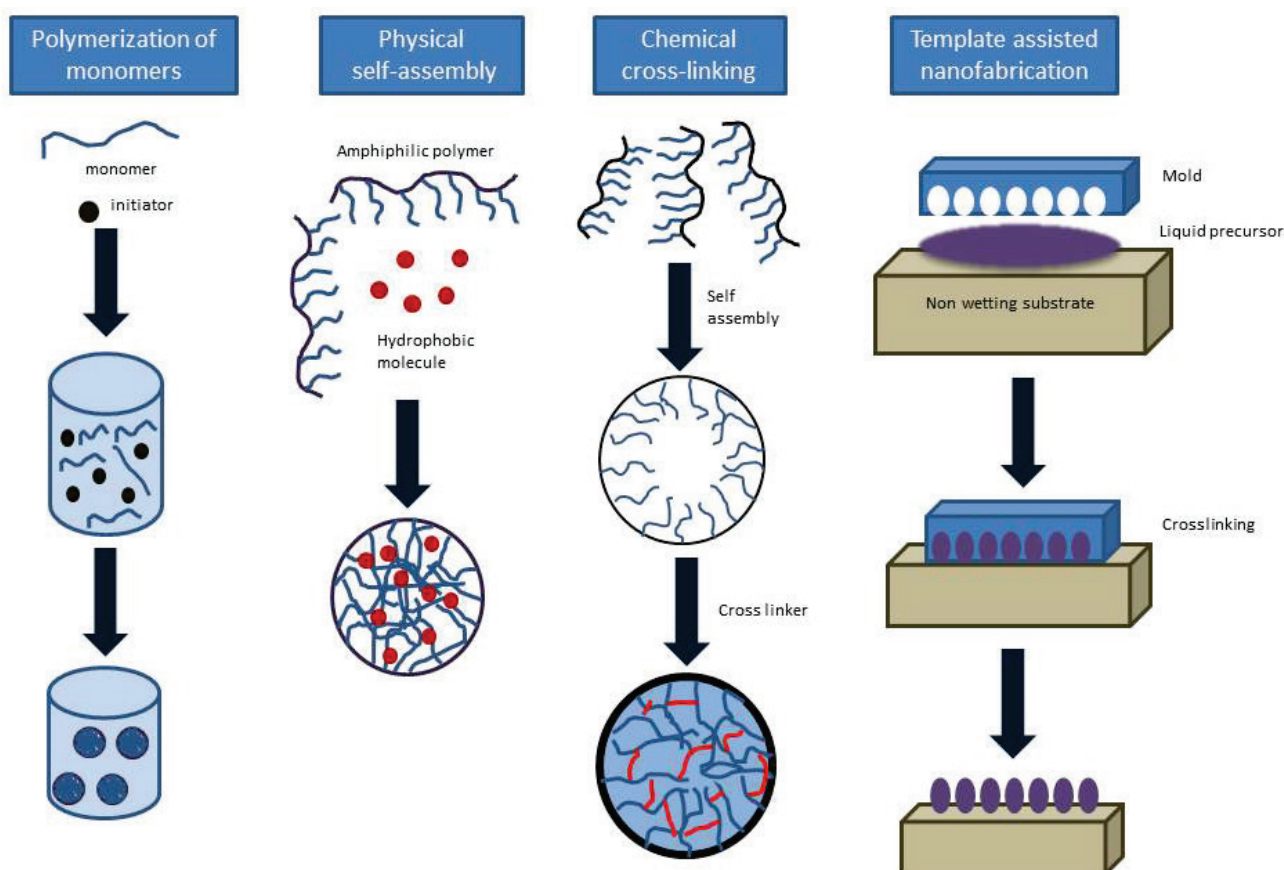

Fig. 2. Schematic representation of different methods for the preparation of nanogels.

Cross-linking of polymers

Another variant of nanogel synthesis is the cross-linking of polymer chains to form covalent bonds (Hennink and van Nostrum 2012). The cross-linking method has been successfully used for the synthesis of various functionalized nanogels for the transport of drugs. For example, this procedure was used to synthesize the first cross-linked cationic nanogel for the transport of polynucleotides (Vinogradov *et al.* 1999). In this case, double-activated PEG was conjugated to branched PEI in an oil/water emulsion.

- Disulphide-based cross-linking

The disulphide bond is stimuli sensitive and biodegradable by biochemical reductants such as

glutathione or thioredoxin I/II. As an example, we can mention nanogels prepared by the cross-linking of polymers containing polyethylene glycol as a hydrophilic unit and pyridyldisulphide as a cross-linkable unit. The addition of catalytic amounts of dithiothreitol reduced a controlled amount of pyridyldisulphide group to thiols located in the polymer matrix, which further exchanged with remaining pyridyldisulphide groups, yielding a cross-linked nanogel. The size of particles can be controlled in this particular case by the amount of dithiothreitol. This system was used for the controlled delivery of doxorubicin (Li *et al.* 2009, Jiwpanich *et al.* 2010, Chacko *et al.* 2012).

- Amino groups involved in cross-linking

A great advantage of using diamines as a cross-linker is their good reactivity with activated esters, isothiocyanides, activated amides or carboxylic acids in the presence of coupling agents. In addition to other nanogels, synthesis of shell cross-linked structures using a diamine cross-linker was developed by (Huang *et al.* 1998). A variety of amphiphilic block copolymers, which contain poly(acrylic acid), were used as the cross-linkable part. For example, polystyrene-*b*-poly(acrylic acid) as an amphiphilic polymer was used for the synthesis of core-shell nanogels. Poly(ethylene glycol)diamine was used as a suitable cross-linker (Huang *et al.* 1998, Joralemon *et al.* 2005, Li *et al.* 2008, Chacko *et al.* 2012).

- Cross-linking using click chemistry

Click chemistry can be used to synthesize nanogels at high yield either by catalyzed or non-catalyzed click reaction. As an interesting example of a core-shell nanogel, we can mention the system based on assembled nanoparticles prepared from amphiphilic diblock copolymer of poly(acrylic acid)-*b*-poly(styrene) in which acrylic acid was coupled with propargylamine using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as a coupling agent. In the following reaction the triple bonds of the propargylamine group react with a suitable azido-containing cross-linker to form a cross-linked shell of the core-shell nanogel (Joralemon *et al.* 2005).

Template-assisted nanofabrication

DeSimone developed (Gratton *et al.* 2007) this new imprinting method, also known as particle replication in non-wettable templates (PRINT), which is suitable for the production of nanogels. Polymeric nanoparticles in the range of tens of nanometers to several micrometers can be prepared using this method. This method allows particle size control, composition, shape, surface functionality and good loading control of pharmaceuticals and biomacromolecules. For example, PEG-based monodispersed swelling particles have been prepared using this method by UV-induced copolymerization of several monomers, such as trimethylolpropane ethoxylate triacrylate, O-(methacryl)-O'-methylpolyethylene glycol and *p*-hydroxystyrene. The master template is created using lithographic techniques. Liquid fluoropolymer is added to the surface of this template. While this fluoropolymer is wetting the template, it is photochemically cross-linked. A form with nanoscale cavities is generated. Due to the low surface energy and high gas permeability, the organic liquid precursor could fill the cavities through capillary action. Indeed, an inter-connecting layer of liquid wetting the area between the cavities is not formed. Nanoparticles prepared using this method have the same precise shape of the master template they were derived from (Rolland *et al.* 2005, Gratton *et al.* 2007, Napier and DeSimone 2007).

Characterization of nanogels

Prior to use, nanogels should be well characterized and the following methods are generally considered suitable for this purpose issue:

Dynamic light scattering

Dynamic light scattering (DLS) is a technique used to determine the size distribution profile of nanoparticles in solutions. During the measurements, light scattering is recorded in a microsecond time scale. The effective hydrodynamic particle radius can be used to measure the effect of the cross-linker and the possible charge of the polymer chains on the size of the formed nanogel. DLS can also be used to measure the swelling of nanogels in different media (McAllister *et al.* 2002, Chen *et al.* 2007). It is worth mentioning that the DLS data should not be overestimated as the DLS measurements in neglecting population of the smaller polymer particles. A combination of analytical methods is often needed to fully understand the characteristics of nanogels.

Scanning electron microscopy

An electron microscopy provides the ability to determine the particle surface as well as its size. Scanning electron microscopy (SEM) can be used to measure particle size from 50-80 nm and to determine the morphological characteristics of nanogels (Somasundaran and Chakraborty 2004).

Circular dichroism

Circular dichroism (CD) serves to detect the optical activity of the resulting product. This method is particularly suitable for detecting chiral molecules that have been inserted into nanogels. Their presence leads to macromolecular structures with a chiral center based on spiral structures that can be detected by CD (Somasundaran and Chakraborty 2004).

Size-exclusion chromatography

Size-exclusion chromatography (SEC) is a method that divides the material according to its size. It is most commonly used to measure the distribution of the nanogel molar mass and the molar mass of individual fractions (Shidhaye *et al.* 2008).

Field-flow fractionation

Field-flow fractionation (FFF) is a separation techniques in which cross-flow is applied to a solution or suspension which is pumped through a long narrow channel. The direction of this cross-flow is perpendicular to the direction of flow. Since FFF can separate polymer material over a wide colloidal size range while maintaining high resolution, it is a unique method in comparison to other separation techniques. Generally, FFF is based on the laminar flow of particles in a solution. Sample components flow at various velocities due to their size/mass and thus separation occurs due to different speed of the components (Giddings *et al.* 1976).

Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) is a technique for sizing particles from approximately 30 to 1,000 nm. This technique combines laser light scattering microscopy with a charge-coupled device camera that enables the visualization and recording of nanoparticles in a solution. NTA is able to identify and track individual nanoparticles moving under Brownian motion and relates the movement to particle size. Both particular size and concentration are measured (Gyawali *et al.* 2017).

Swelling studies of nanogels

Swelling is the most important property of nanogels and is characterized by measuring their capacity to absorb water or an aqueous solution. The easiest way to determine the kinetics and swelling equilibrium is to measure weight, with the degree of swelling being calculated from the weight portion of the swollen nanogel and the initial weight. The swelling of the nanogel is influenced by the following factors: type and composition of the monomer, cross-link density, pH, temperature and ionic strength (Kopeček 2002, Vinogradov *et al.* 2005).

Nanogels as drug carriers

Nanogels are suitable for the delivery of various drugs from hydrophobic to hydrophilic ones. There are several methods described in the literature for the encapsulation or attachment of drugs. The following section describes the commonly used approaches:

Covalent conjugation

This method leads to the formation of a covalent bond between suitable groups of the drug and the functional groups of the nanogel. The most widely used covalent bonds are stimuli-sensitive, thus enabling the release of the drug at the place of interest within the body. A pH-responsive hydrazone bond between doxorubicin and the methacrylamide polymeric nanogel was used recently for solid tumor drug delivery and activation (Chen *et al.* 2017). Furthermore, biomacromolecules can be also covalently bound to nanogels. For example, enzymes are attached through a two-step reaction. The first step is the reaction of the enzyme with N-hydroxysuccinimidoacrylate under mild conditions. This reaction generates double bonds on the enzyme surface. The second step is *in situ* polymerization with acrylamide as the monomer, *N,N'*-methylenebisacrylamide as the cross-linker and *N,N,N',N'*-tetramethylethylenediamine/ammonium persulfate as the initiator (Yan *et al.* 2006). Alternatively, polyacrylamide nanogels with incorporated modified α-chymotrypsin can be prepared by copolymerization in an inverse micropolymerization reaction (Khmelnitsky *et al.* 1992). Nanogels containing covalently-bound proteins can increase their thermostability and plasma half-life.

Direct addition method

In this method, the drug is dissolved together with the monomer in the aqueous phase of the emulsion before the synthesis of the nanogel. The drug is therefore encapsulated in the nanogel structure during its formation by hydrophobic or electrostatic interaction. Using this procedure, aspirin-containing nanogels were prepared by photoisomerization using a solution of the aspirin salt dispersed in a solution of linear dextran containing *N*-(6-aminohexyl)-4-[4-hydroxyphenylazo]-benzamide substituent attached *via* an amide bond. The first step of the synthesis was the preparation of the hydrophobic substituent, while the second step was the reaction of the substituent with dextran. The nanogel was then formed through non-covalent self-aggregation induced by photoisomerization (Patnaik *et al.* 2007).

Soaking method

This method is useful in the case of amphiphilic nanogels containing hydrophobic moieties such as cholesterol. The drug can be introduced by simply dipping the nanogels in a supersaturated solution of the drug. For example, this method was used to synthesize indomethacin-carrying nanogels (Sahiner *et al.* 2007).

Passive and active targeting

Passive targeting using nanogels in neoplastic disease treatment is, as in the case of other nanomedicines, based on an enhanced permeation retention effect (EPR effect). The vessels nourishing the

tumor are poorly formed, leading to large gaps and thus increased permeation by macromolecular delivery systems in the range 20-200 nm occurs. On the other hand, lymphatic drainage is poor due to the rapid growth of the tumor, which leads to nanoparticles remaining in the tumor tissue. Thus, the EPR effect enables the solid tumor tissue to be targeted using size-controlled nanogels. In this regard, tailor-made nanogels with controlled size should present significant advantages for therapies targeting solid tumors and metastases. Moreover, the size of the nanosystem determines the internalization route and speed (Chacko *et al.* 2012, Rigogliuso *et al.* 2012).

To obtain active targeting, nanogels have to be functionalized with suitable molecules that are capable of recognizing and binding to specific receptors on the targeted cells. These receptors should be primarily expressed on the targeted cells. Generally, large macromolecules (e.g. monoclonal antibodies) or small molecules (e.g. oligopeptides or oligosaccharides) may be used as targeting ligands to the receptors on the targeted cells. The significance of this strategy can be demonstrated, for example, in DNA drug delivery systems in which the sequence of the cell-penetrating peptide leads to significant differences in DNA delivery efficiency (Roy *et al.* 2009). This work demonstrated the importance of post-assembly surface modification of the drug-loaded delivery system. If the TAT peptide was attached to the preformed assembly, delivery transfection efficiency was about 25 % higher than when the TAT peptide was attached to the delivery system before complexing with DNA (Chacko *et al.* 2012). Encapsulated paclitaxel in nanogels was actively targeted to liver, breast or prostate tumors using galactosamine, transferrin, anti-HER2 or fragments of mAbs (anti-HER2 scFv F5) as moieties selective for those cancer cells (Park *et al.* 2001, Sahoo *et al.* 2004, Xu *et al.* 2005, Liang *et al.* 2006, Steinhauser *et al.* 2006, Sun *et al.* 2008).

Application

Nanogels in cancer therapy

Many drug delivery systems, mainly nanoparticles, liposomes and soluble polymer systems, have been investigated to overcome the limitation of standard chemotherapeutics such as narrow therapeutic window, poor solubility and cytotoxicity to normal tissues (Zhang *et al.* 2016). Nanogels were used in the treatment of cancer and as carriers of the following low-molecular weight drugs: doxorubicin, cisplatin, 5-fluorouracil,

temozolomide, andriamycin, neocarzinostatin etc. (Morimoto *et al.* 2006, Shidhaye *et al.* 2008, Sharma *et al.* 2016). Nanogels containing doxorubicin are often studied in the treatment of cancer in the form of pH- and temperature-responsive nanogels based on maleic acid poly-(*N*-isopropylacrylamide) polymers. Doxorubicin is released from these nanogels following a slight decrease in pH or through temperature stimulus (Sharma *et al.* 2016). In addition, a chitin-based doxorubicin-bearing nanogel was studied in the treatment of prostate, breast, lung and liver cancer. Table 2 summarizes the different types of nanogels used to treat cancer.

Nanogels for delivery of protein and peptide

Nanogels can also be used as carriers of proteins and peptides to the target site. Artificial nanogels are one of the possible carriers used to transport various proteins (Sharma *et al.* 2016). Different types of nanogels used in the controlled delivery of protein and peptides are listed in the Table 3.

Nanogels for gene and antisense delivery

Progress in recent years in the field of gene and antisense therapy has led to the implementation of safe, effective drug delivery systems. Various viral and non-viral vectors have been tested for the delivery of oligonucleotides. However, due to the limitations of viral vectors (immune response and toxicity), significant emphasis has also been given to non-viral vectors. Taking into account the limiting factors of classical non-viral vectors, namely correct size achievement and poor stability, a huge effort was made to develop polymeric nanogels of a size less than 200 nm that were able to form monodisperse complexes with DNA or oligonucleotides. The nanogel-oligonucleotide complex was shown to increase the physico-chemical stability of the oligonucleotide and to enable targeted delivery of the oligonucleotide (Shidhaye *et al.* 2008). McAllister *et al.* reported the synthesis of monodisperse and non-toxic cationic nanogels capable of forming stable complexes with oligonucleotides. The system was prepared through the inverse microemulsion polymerization of 2-acryloxyethyltrimethylammonium chloride with 2-hydroxyethylacrylate and poly(ethylene glycol)diacrylate. The quaternary ammonium group of the 2-acryloxyethyltrimethylammonium chloride-based monomeric unit promoted pH-independent condensation of the oligonucleotide *via* electrostatic dissociation between the phosphate group of the oligonucleotide and the quaternary ammonium group (McAllister *et al.* 2002).

Table 2. List of nanogels investigated in cancer treatment

Nanogel constitution	Type of nanogel	Drug used	Applications	References
Poly(N-isopropylacrylamide-co-metacrylic acid-co-N,N'-methylenbisacrylamide-co-O-(methacryl)-O'-methylpolyethylene glycol)	Temperature- and pH-responsive nanogel	Cisplatin	Breast cancer therapy	Peng *et al.* 2013
Poly(N-isopropylacrylamide-co-polyethylenimine-co-N,N'-methylenebisacrylamide)	Temperature- and pH- responsive nanogel	5-fluorouracil	Mastocarcinoma therapy	Zhu *et al.* 2017
Acetylated chondroitin sulphate	Self-assembled by hydrophobic interaction	Doxorubicin	Cervical cancer	Park *et al.* 2010
Poly (acrylic acid-co-N,N'-methylenebisacrylamide) filled with hydroxypropylcellulose	Temperature- and pH-responsive nanogel	Temozolomidine	Melanoma	Wu *et al.* 2010
Poly(N-isopropylacrylamide-co-butylacrylate-co-N,N'-methylenebisacrylamide)	pH-responsive	Methotrexate	Breast cancer, lung cancer, leukemia and lymphoma	Singka *et al.* 2010
Disulphide cross-linked heparin nanogel	Reducible nanogel	Heparin	Induction of apoptosis of melanoma cells	Bae *et al.* 2008
Self-assembly pullulan-based nanogel with folic substituents	pH-responsive	Doxorubicin	Cervical cancer	Kim *et al.* 2008

Table 3. List of nanogels investigated for protein and peptide delivery.

Nanogel constitution	Type of nanogel	Applications	References
Self-assembled cholesterol-bearing glycogen filled with cyclodextrin	Nanogel with thermally stable properties suitable for preservation of proteins	Thermal stabilization of enzyme for biomedical use, pharmaceutical and cosmetic application	Takahashi *et al.* 2011
Poly(methylacrylic acid-co-N,N'-ethylenebisacrylamide)-coated Fe$_3$O$_4$ nanoparticles	pH- and temperature-responsive	Controlled delivery of α-chymotrypsin using a magnetic field	Hong *et al.* 2008
Self-assembled cholesterol-bearing pullulan	Stimuli sensitive (heat, light)	Controlled delivery of bone anabolic agents, e.g. recombinant hormones, and cytokines	Nomura *et al.* 2003

Nanogels as vaccine delivery systems

The induction of a specific immune response against cancer cells is a highly achievable goal in the immune therapy for cancer. Complex vaccines of hydrophobic polysaccharide nanogels containing truncated oncoprotein complexes can induce strong cellular and humoral immune responses against HER2-expressing cancers. Following exposure to the cholesterol-bearing pullulan-HER2 nanogel complex, dendritic cells such as bone marrow-derived APC were capable of eliciting a host immune response through stimulation of the proliferation of CD4$^+$ T cells and CD8$^+$ T cells (Morimoto *et al.* 2006).

Nanogels for therapy of Alzheimer's disease

The formation of fibrils by amyloid β-protein is considered a key step in the pathology of Alzheimer's disease. Inhibiting the aggregation of amyloid β-protein represents a promising approach for therapy. Nanogels composed of a polysaccharide pullulan backbone with cholesterol hydrophobic moieties (cholesterol-bearing pullulan) as an artificial absorber of amyloid proteins can be used to inhibit the formation of amyloid β-protein-(1-42) fibrils with marked anti-amyloidogenic activity. 6-8 molecules of amyloid β-protein-(1-42) can be incorporated per one. Nanogels composed of amino-group modified cholesterol-bearing pullulan with positive charges under physiological conditions showed a superior inhibitory effect than unmodified cholesterol-bearing pullulan possibly due to electrostatic interactions between the amino-group modified nanogel and the amyloid β-protein, which can be important in the inhibition of

fibril formation. In addition, these nanogels can protect PY12 cells from amyloid β-protein toxicity (Kudva *et al.* 1997, Ikeda *et al.* 2006).

Conclusions

Several synthetic approaches and drug molecule entrapment approaches for the advanced synthesis of nanogel-based drug carriers have been described to review the relationship between the structure and physico-chemical and biological characteristics of nanogel systems. Indeed, the application of these systems as nanomedicines, especially for therapy of neoplastic diseases and other diseases, has been described in detail, showing the potential of these nanogel systems. Taking the recent development of nanogels in consideration, we believe that there is huge potential in future research and clinical evaluation of tailored nanogels with smart properties based on the controlled physico-chemical properties of system, tailor-made degradation and advanced functionalities. We can unequivocally conclude that nanogels are highly interesting polymer carriers suitable for further preclinical development.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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