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Nanogel Engineering by Associating Polymers for Biomedical Applications
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8.1 Introduction

Nanogels (nanometer-sized hydrogel nanoparticles, diameter <100 nm, Figure 8.1) have attracted increasing interest in recent years because of their potential applications in drug-delivery systems (DDS) [1–3]. Unlike the nanocarriers usually used for DDS, such as nanospheres, which have a densely packed core structure, nanogels can take up a large amount of water and can incorporate bioactive compounds including drugs, proteins, and DNA within their nano-scale polymer networks in a relatively stable manner. Furthermore, molecules that are complexed with nanogels can be released by a change in the network structure or a gel-phase transition. These properties of nanogels, in comparison with other nanocarriers such as liposomes and polymer micelles, enable controlled release of the bioactive compounds by phase transition and a change in the network structure in response to specific environmental stimuli.

Nanogels can be classified as either chemically crosslinked nanogels or physically crosslinked nanogels. Chemically crosslinked nanogels are formed by crosslinking of polymers via covalent bonding. In contrast, noncovalent bonds (i.e., hydrogen bonds, ionic bonds, and hydrophobic interactions) link the polymers in physically crosslinked nanogels [4]. Typically, chemically crosslinked nanogels are synthesized under diluted conditions by a crosslinking reaction of polymers modified with reactive groups such as vinyl and thioc groups. Nano- or micro-emulsion polymerization methods are often used to obtain nanogels with a well-controlled size. A variety of new synthetic techniques have been reported recently, including the block copolymer crosslinking method in which micelles composed of amphiphilic block copolymers or ployion complexes are crosslinked [5–7], the nano-template method, which exploits the internal water phase of liposomes [2] or the surface of silica or gold nanoparticles as templates for nanoreactions [8, 9], and the lithography-based top-down method [10, 11]. Various chemical crosslinking reactions have now been developed, including carbodiimide-mediated amide bond crosslinking [5–7], quaternization of amino groups [12], “click” chemistry [13], and...
photo-crosslinking [14, 15]. Meanwhile, nanocarriers bearing disulfide bonds have been shown to be useful for efficient gene delivery [16]. Since many reports and excellent review articles have described the methods used to synthesize chemically crosslinked (non-associating polymer-based) nanogels [1], this method will not be discussed in detail here.

Noncovalent interactions, such as hydrogen bonds, van der Waals forces, and electrostatic and hydrophobic interactions are also available to prepare nanogels. However, it is difficult to obtain stable physically crosslinked nanogels with controlled sizes using noncovalent interactions because the bonds are relatively weak. In this chapter, we summarize the preparation and characteristics of associating polymer-based nanogels and discuss recent studies describing the applications of nanogels as DDS carriers. We also discuss recent advances in the development of nanogel-based biomaterials and their applications to regenerative medicine, along with our research achievements.

8.2 Preparation of Associating Polymer-Based Nanogels

8.2.1 Hydrophobically Modified Polysaccharide

In a recent study we reported the first physically crosslinked nanogels prepared by controlled association of hydrophobically modified polymers in a dilute aqueous solution [17]. Cholesterol-bearing pullulans (CHP), which are composed of hydrophilic pullulan partially modified with cholesteryl groups, self-assemble in an aqueous solution and form stable nanogels with a diameter of 30 nm (Figure 8.1) [18]. The association of hydrophobic cholesteryl groups provides crosslinking points via hydrophobic interactions. Nanogel formation is regarded as one of the most interesting functions of the associating polymers. The closed association of polymers...
at the nanosize scale is governed by many factors, including the concentration of the associating polymers, the structure and concentration of the crosslinking points, and the characteristics of the associating polymers. Self-assembling nanogel formation from hydrophobically modified polymers has also been demonstrated with other associating polymers such as poly(aminoacids) [19] and hydrophilic synthetic polymers onto which associating groups are partly grafted [20, 21].

As a backbone polymer, polysaccharides (Figure 8.2) are often used to form nanogels because of their excellent biocompatibility and availability, which are favorable for DDS applications. Deoxycholic acid-modified glycol chitosan [22],

![Structures of the parent polysaccharides commonly used for nanogel formation.](image)
deoxycholic acid-modified heparin [23], bile acid-bearing dextran [24, 25], and cholesterol-modified dendritic dextrin [26] have been used as polysaccharides for nanogel formation. The effects of the hydrophobic structures and of the degree of substitution on the physicochemical properties of the resulting nanoparticles have been investigated in detail, leading to design rules with respect to the hydrophobic moieties. For example, cholesterol-modified highly branched mannan (CHM) formed highly hydrated nanogels in water through the association of cholesteryl moieties in a manner similar to that of other hydrophobically modified linear polysaccharides such as CHP [27]. A comparative study of the CHM and CHP nanogels revealed that the structure and level of hydration of the polysaccharide chain significantly affect the microscopic structure of the self-aggregates and the microviscosity of the hydrophobic domain within the nanogels [27]. We have also reported a novel amphiphilic nanoball in which a cholesterol group was introduced to enzymatically synthesized glycogen (ESG) [28]. ESG is a highly branched \((1 \rightarrow 4)(1 \rightarrow 6)\)-linked \(\alpha\)-glucan and is a monodisperse spherical hyperbranched nanoparticle. Cholesterol-modified ESG assembled into a structure containing a few molecules in water to form a cluster of nanogels, which has great potential as a new building block for nanogel biomedical engineering.

One of the most significant characteristics of polysaccharide nanogels is their ability to incorporate proteins into nanogels and form stable complexes through hydrophobic interaction [29]. They can also protect proteins from irreversible aggregation to provide colloidal and thermal stability and release proteins in active forms from nanogels in response to external stimuli. These functions are described later in detail.

8.2.2 Photoresponsive Molecule-Modified Polysaccharides

In recent years, various stimulus-responsive materials have been developed and applied in biological and medical fields as DDS and for tissue engineering [31–37]. Stimulus-responsive nanogels have also attracted much attention because nanogels show an unusually rapid response to microenvironmental stimuli such as temperature and pH because of their nano-scaled dimensions. Associating polymers can be used to prepare stimulus-responsive nanogels because of the formation of noncovalent crosslinks, which can be easily modified by external stimuli. To date, several stimulus-responsive nanogels have been developed by introducing stimulus-responsive molecules to the polymers.

A photoresponsive nanogel was synthesized by substituting pullulan with a spiropyran molecule, a hydrophobic group that changes the polarity of the molecule in response to light and heat [30]. Spiropyran molecules which isomerize from hydrophobic spiropyran to hydrophilic merocyanine under photo- and thermo-stimulation are widely used to control the structure and function of biomaterials by exposure to light. Spiropyran-modified pullulan forms \(~75\) nm nanogels in water. The assembly is controlled by changing the amphiphilicity of the spiropyran molecule due to exposure to photo-irradiation or heat (Figure 8.3). In addition,
refolding of the chemically denatured proteins after dilution of the protein solution is facilitated by photo-irradiation. Photoresponsive nanogels also act as novel photoresponsive artificial molecular chaperones.

8.2.3 Thermoresponsive Polymer-Grafted Polysaccharide

Thermoresponsive materials with low critical solution temperatures (LCSTs) in aqueous media have received much attention [38]. For example, some researchers have prepared thermoresponsive nanogels that enable heat-induced association and dissociation of polysaccharides partially grafted with short poly(N-isopropylacrylamide) (PNIPAM) chains [39]. These polymers readily dissolve in water at room temperature. Above the LCST, PNIPAM-g-polysaccharides form nanogels that are physically crosslinked by the hydrophobic nanodomains generated by dehydration of PNIPAM. Biocompatible heat-induced nanogels have also been obtained by grafting poly(2-isopropyl-2-oxazoline) (PIPOZ) [40], which is a biocompatible, thermoresponsive crystalline molecule, onto a polysaccharide (Figure 8.4). The assembly of the polymers is driven by the crystallization of PIPOZ chains following heat-induced dehydration of the chains in water heated above the LCST.

A dual stimuli-responsive nanogel that responds to changes in environmental temperature and redox potential has also been reported [41]. That nanogel was developed by associating a redox-responsive polymer containing a thiol group with the terminus of the PNIPAM chain, which forms a disulfide bond after nanogel formation. The formation of the nanogel is controlled by heating and cooling of the solution because the hydrophilic–hydrophobic transition of the PNIPAM side chains is temperature-dependent. Assembling the PNIPAM chain with a thiol group at its terminus provides nanogels that are chemically crosslinked via a disulfide bond after
the nanogel is formed at a temperature above the LCST. Even after cooling the temperature to below the LCST, the structure of the nanogel is maintained as a swollen hydrophilic nanogel. In the presence of a reducing agent, the nanogel can be reduced to its thiol group to afford a dual-responsive nanogel that responds to both temperature and redox potential.

8.2.4 Metal–Ligand Modified Polysaccharides

Redox-sensitive nanogels have been found to be useful for efficient gene delivery [16]. As described above, redox-responsive nanogels can be obtained by using disulfide bonds as a crosslinking point [41]. More recently, redox-sensitive metal coordinative-crosslinked nanogels were prepared by introducing a metal–ligand to a hydrophilic polysaccharide and CHP (ImP and ImCHP, Figure 8.5) [42]. Metal-complexing and metal-containing polymers are fascinating research targets, particularly because such materials have properties that differ from their individual organic and inorganic components. These polymers offer a wide variety of applications that range from filtration to catalysis. The coordinating ability of the metal within the polymer chain enables their application in environmental metal sensing and as building blocks for supramolecular structures. We used metal ligands to prepare novel redox-responsive

![Figure 8.4](image1.png) Nanogels enable heat-induced association of polysaccharides partially grafted with short poly(N-isopropylacrylamide) (PNIPAM) chains.

![Figure 8.5](image2.png) Structures of the metal–ligand-modified polysaccharide, CHP, and the metal-coordinative crosslinked nanogel.
nanogels [42]. As a metal–ligand, imidazolyl groups were conjugated with pullulan. The imidazolyl group has a high affinity for divalent transition metal ions and forms a complex. Complexation of more than one ligand group per metal ion can link two or more polymer chains at a metal-centered crosslink. In addition, the redox couple of Co(II) and Co(III) afforded this metal coordinative nanogel system with good redox sensitivity.

8.2.5 Siloxane-Modified Polysaccharides

The development of organic/inorganic hybrid nanomaterials is a fascinating research target in the fields of biosensing, bioimaging, and DDS [43, 44] because of the specific functionality of the organic part and the structural stability of the inorganic part. For example, hybrids consisting of polysaccharides with calcium phosphate and hydroxyapatite nanoparticles have been prepared [45, 46]. The hybrid nanogels are useful as intracellular protein carriers because of their excellent biocompatibility and biodegradability, in addition to their mechanical stability. In another example, hybrid nanogels were prepared by condensation of inorganic silanol groups (sol–gel reaction) grafted onto polysaccharides at ambient temperature and pH without any catalysts or organic solvents [47]. The hybrid nanogels were made rigid upon covalent crosslinking with inorganic siloxanes, which provides an additional platform for mineralization with other silane-coupling agents, titania and hydroxyapatite, for example. These stable hybrid nanogels are promising candidates for controlled-release DDS. In fact, using hybrid nanogels based on PNIPAM gels and tailored nanoporous silica, the carried drug can slowly diffuse out of the porous channels via a nanodiffusion mechanism [48].

8.2.6 Protein-Crosslinked Nanogels

Biomolecules such as oligopeptides [49], proteins [50], antibodies [51], and oligonucleotides [52] have been widely used as crosslinkers to prepare macro-scale hydrogels. One of the main advantages of biomolecule-crosslinked hydrogels is that their response to stimuli is controlled and modulated by the complexed biomolecule [53]. Despite the promising utility of crosslinked biomolecules, there are few examples of biomolecule-crosslinked nanogels, perhaps because of the difficulty in conjugating the biomolecules with polymer chains in an aqueous environment. We recently prepared protein-crosslinked nanogels by introducing vitamin B₆ (pyridoxal) to hydrophilic polysaccharide [54]. One of the important chemical characteristics of the pyridoxal moiety is that it acts as an active aldehyde to form a Schiff base between the amine and the pyridoxal formyl group. By using the Schiff base, the pyridoxal-modified polysaccharides were held together to form nanogels that crosslinked with lysozyme containing six lysine residues (Figure 8.6). The pH-dependence of Schiff base formation was exploited to construct a pH-sensitive nanogel system. These pH-sensitive hybrid nanogels, upon Schiff base
formation with various biomolecules, are useful nanocarriers in DDS and could be used for cytosolic protein delivery.

8.3 Functions of Self-Assembled Nanogels

8.3.1 Nano-Encapsulation of Proteins by the Nanogel: Nanogels as Macromolecular Hosts

The design of a macromolecular host with a nano-cage that can bind macromolecular guests, such as proteins, and the ability to control uptake and release of the guest molecules are indispensable properties of effective DDS of biomacromolecules. The development of a macromolecular host that protects the accommodated biomolecule, particularly for proteins, DNA, and RNA, is essential because such molecules undergo rapid degradation by endolysosomal proteases and nucleases. One of the most notable characteristics of associating polymer-based nanogels is their dynamic properties, which enable them to spontaneously trap proteins in the relatively flexible nano-scale hydrogel matrix and protect them. For example, CHP nanogels were reported to selectively interact with various proteins, primarily through hydrophobic interactions [29, 55]. The nanogel–protein complexes showed excellent colloidal stability without any precipitation. One CHP nanogel complexed with approximately one bovine serum albumin ($M_w$ 66 000), two $\alpha$-chymotrypsin ($M_w$ 25 000), two
myoglobin (M_w 17 800), four molecules of cytochrome c (M_w 12 500), and five molecules of insulin (M_w 5735). The maximum number of protein molecules complexed by CHP nanogels depends on the molecular weight (or size) and hydrophobicity of the protein. This is an interesting example of host–guest interactions in a macromolecular system.

In most cases, the activity of an enzyme is lost following complexation because of the induction of conformational changes in the enzyme due to the preferred hydrophobic interactions between the protein and the cholesterol groups of CHP. However, we found that complexation with a nanogel afforded high enzyme activity and thermal stabilization of lipase [56]. Lipases are key biocatalytic enzymes involved in the synthesis of bioactive molecules and biodegradable biopolymers. The thermostability of the lipase complex increased because the denaturation temperature of lipase increased by more than 20 °C after complexation with CHP nanogels. Lipase denaturation and aggregation upon heating was effectively prevented by complexation with CHP nanogels. Complexation with CHP nanogels also protected lipase from lyophilization-induced aggregation. Thus, nano-encapsulation with CHP nanogel as a macromolecular host is a useful method to achieve colloidal and thermal stabilization of an unstable enzyme.

8.3.2 Artificial Molecular Chaperones

In living systems, natural molecular chaperones such as GroEL/ES aid protein folding by preventing the aggregation of denatured proteins [57]. In general, native proteins partially unfold in response to stressors, such as heat, which expose the hydrophobic groups to the outer surface. Hydrophobic interactions between the exposed hydrophobic chains cause aggregation and the formation of insoluble aggregates in a (usually) irreversible manner. Molecular chaperones selectively trap the folding intermediate proteins or heat-denatured proteins via hydrophobic interactions to prevent irreversible aggregation. Then, with the aid of ATP and another co-chaperone, the chaperone releases the folded (refolded) proteins.

The use of polysaccharide nanogels as a macromolecular host can simulate these molecular chaperone systems because of the dynamic properties of the associating polymers [58]. As described above, CHP-based nanogels can interact with proteins. More interestingly, many enzymes and proteins can be captured within the nanogel network in a heat-denatured form above their denaturation temperatures [59]. Because the nanogel network is comparable with that of proteins, the nanogel can hold a protein molecule within a segregated nanomatrix. The ability of part of the nanogel network to isolate protein molecules is advantageous for stabilizing proteins, because protein–protein interactions and aggregation can be eliminated. Nanogels also prevented protein aggregation (e.g., carbonic anhydrase and citrate synthase) by forming nanogel–protein complexes during protein refolding after chemical denaturation by urea or guanidium hydrochloride [60, 61].

Fluorescence correlation spectroscopy (FCS) was recently used to evaluate the thermodynamic properties of the interaction between nanogels and proteins at
variable temperature [62], although preliminary thermodynamic studies were conducted using isothermal titration calorimetry at a single temperature [63]. FCS has been used to monitor the binding process by determining the diffusion of fluorescently labeled molecules. The van’t Hoff plot showed that the CHP nanogels strongly complexed with heat-denatured proteins. The increased hydrophobicity of the denatured, unfolded protein may favor complexation with amphiphilic hydrogel nanoparticles over complexation with the completely folded native protein. Other thermodynamic parameters suggest that under the conditions studied the complexation is driven in an entropic rather than an enthalpic manner, indicating that the complexation is driven by hydrophobic interactions between proteins and nanogels.

Using a series of cyclodextrins (CDs), the polysaccharide nanogel system can simulate the release process controlled by molecular chaperones to afford folded active proteins. CHP nanogels can be dissociated by the addition of CD [64]. CD formed inclusion complexes with cholesteryl groups and dissociated the hydrophobic crosslinking points in the nanogel network. Indeed, we have reported the molecular chaperone-like activities of a CHP nanogel system using enzymes such as bovine carbonic anhydrase and porcine citrate synthase [59, 65, 66]. The nanogel–CD system has some superior features compared with other artificial chaperone systems. Similar two-step systems employing surfactants and CDs [67] are referred to as “artificial chaperone” techniques. However, it can be difficult to completely remove the surfactants, which may denature proteins after treatment with such systems. These polymers appear to act as a hydrophobic buffer by blocking the exposed hydrophobic surface on the denatured protein and thus preventing aggregation. In this polymer system, very strong binding to the intermediate would prevent folding to the native conformation. The current nanogel system can provide a delicate balance for the various folding processes and afford a high refolding efficiency with the broad specificity of natural molecular chaperones. In fact, the chaperone-like activity of CHP nanogels has been demonstrated in the refolding of acid-denatured green fluorescent protein (GFP) [68], the renaturation of the inclusion body of a recombinant protein [69], and the suppression of Aβ toxicity in primary cortical cultures and in microglial cell cultures [70].

8.3.3 Nanogel Chaperones in Cell-Free Protein Synthesis

Cell-free protein synthesis is a promising technique for the rapid production of proteins. However, the application of cell-free systems requires the development of artificial chaperones that prevent protein aggregation and support its correct folding. We reported that the CHP nanogel acted as an artificial chaperone in cell-free protein synthesis of GFP [71]. In this cell-free GFP system, GFP spontaneously folded with a relatively high yield in the absence of molecular chaperones so that the CHP nanogel–CD system did not improve folding efficiency. More recently, we showed that the nanogel-based artificial chaperone improves the folding efficiency of rhodanese produced in a cell-free system [72] (Figure 8.7). Rhodanese normally aggregates rapidly, but it was successfully expressed in the presence of the nanogel and folded
to the enzymatically active form after the addition of CD. The general applicability of the nanogel-based molecular chaperone system was also demonstrated in the cell-free synthesis of 10 water-soluble proteins and it was concluded that the nanogel enables the high expression of proteins with a high tendency for aggregation [72].

8.4 Application of Polysaccharide Nanogels to DDS

Nanogels can be used as carriers in DDS by efficiently trapping not only hydrophobic and hydrophilic low-molecular-weight drugs, but also high-molecular-weight entities, including DNA, siRNA, peptides, and proteins, within the network [1–4, 17]. For example, amphiphilic nanogels with hydrophobic regions have been used for the incorporation and release of insoluble anticancer drugs. In this section, we describe...
the biomedical applications of polysaccharide nanogels, particularly for the purpose of protein and nucleic acid delivery.

8.4.1 Protein Delivery

Biologically active proteins such as hormones, cytokines, growth factors, and antigenic proteins are effective for the treatment of various diseases. The development of technologies capable of efficiently delivering these proteins into the cells is also important in the fields of regenerative medicine and immunotherapy. However, there remain several issues to be overcome, including the instability of the carrier–protein complexes and difficulties in suppressing inactivation, aggregation and controlled release of proteins, which are less stable than low-molecular-weight drugs. The activity of molecular chaperones is an important concept that has led to significant breakthroughs in DDS, particularly for protein or peptide delivery [73, 74].

The main benefits of nanogels as protein carriers were recognized mainly in the field of immunotherapy for the treatment of cancer and HIV by stimulating or activating autoimmune function. Immunotherapy with cytokines and cancer vaccines is an important therapeutic modality because such treatments have minimal side effects and are capable of controlling the growth, recurrence, and metastasis of cancer cells over a prolonged time. CHP nanogels can form stable complexes with the protein antigens used to induce specific immune responses against tumor cells resulting in effective cancer immunotherapy [75–78]. For example, when CHP nanogels containing HER2 protein antigen (a cancer-related gene product) were subcutaneously injected into mice with cholangiocarcinoma, antitumor killer T cells and antibody-producing T helper cells were efficiently induced. A preliminary phase I clinical trial demonstrated the effect of the nanogel system, especially for treatment of esophageal cancer. Since then, CHP nanogels have been found to be effective as controlled-release carriers of interleukins for cytokine immunotherapy of cancer [79].

More recently, it was shown that cationic nanogels are effective carriers of mucosal (intranasal) vaccines [80]. Mucosal vaccines are expected to represent a new generation of vaccines that can prevent infection against pathogens, such as influenza virus, AIDS virus, and norovirus, which infect via the mucosal surfaces. A nontoxic subunit of Clostridium botulinum type A neurotoxin (BoHc/A) administered intranasally with a cationic nanogel adhered continuously to the nasal epithelium and was effectively taken up by the mucosal dendritic cells after its release from the cationic nanogel. Moreover, intranasally immunized tetanus toxoid with cationic nanogel induced systemic and mucosal immune responses. These results indicate that cationic nanogels can be used as a universal protein-based antigen-delivery vehicle for adjuvant-free intranasal vaccination.

The intracellular delivery of exogenous proteins is a topic of growing interest in bioscience and for medical applications [81]. Cationic liposomes [82, 83], cationic microparticles [84–86], and amphiphilic peptides containing protein transduction domains (PTD) [87] have been used as carriers for intracellular delivery. However, these DDS generally suffer from low delivery efficiency and poor specificity for a
particular cell type. We demonstrated that the intracellular protein delivery could be efficiently done using cationic nanogels with chaperone-like functions. For example, ethylenediamine was introduced to the CHP nanogel to form positively charged nanogels \( \sim 30 \text{ nm} \) in size [73]. The cationic nanogels strongly interacted with a wide range of proteins to form stable nanogel–protein complexes, which were efficiently introduced into HeLa cells. One advantage of nanogels is that they can form a colloidal complex with a protein with an overall complex size of several tens of nanometers, which is suitable for effective intracellular uptake. Indeed, it was shown that nanogels are capable of delivering an active enzyme (β-galactosidase) into the nucleus [73]. Protein-coated quantum dots (QDs) have also been effectively delivered into cells and hence can be used as an imaging reagent [88, 89]. We have also reported a new nanogel with a targeting function aimed at enhancing specific delivery via a ligand-conjugated nanocarrier (cell-specific peptide (Arg-Gly-Asp; RGD)-modified nanogel) [90].

8.4.2 Nucleic Acid Delivery

The gene-delivery system is an essential technology in both basic research and in clinical gene therapy [91]. Polymer-based carriers have received extensive attention because they are easier to prepare and have less toxicity than viral carriers. In particular, polysaccharide-based gene-delivery systems such as cationic dextran [92], cationic pullulan [93], cationic cycloamylose [94], chitosan [95], and schizophyllan [96] have been developed because of the low toxicity and high biocompatibility of the constituents. RNA interference has emerged as a new therapeutic pathway for delivering functional RNA, such as siRNA, to target cells. We reported a new siRNA-delivery system based on a functional cycloamylose nanogel [97]. Cholesterol-bearing cationic cycloamylose also formed nanogels by self-assembly in water. The nanogels formed 20–40 nm nanoparticles that bound to 21 bp siRNA. The complexes exerted an RNA-interfering effect on SCC7-GL3 cells stably expressing the luciferase gene.

We recently developed a hybrid gene-delivery system comprising a polysaccharide-based cationic nanogel and phospholipase A\(_2\) (PLA\(_2\)) that was capable of endosomal disruption by hydrolyzing membrane phospholipids [98]. A cationic nanogel formed ternary complexes with PLA\(_2\) and pDNA by hydrophobic and electrostatic interactions. Both pDNA and PLA\(_2\) were internalized into cells by the nanogel. The ternary complex can disrupt the membrane when delivered into cells and triggers the release of pDNA from the endosome to the cytoplasm. Co-delivery of PLA\(_2\) and pDNA using a nanogel may address an important issue in polymer-based gene-delivery systems—the need to overcome endosomal disruption produced by the polyclion complex.

8.5 Integration of Nanogels

Effective control of the structure of the crosslinking points and the nano-domains in macro-scale hydrogels, which have been widely used as functional materials in
biotechnological and biomedical applications, is an important focus of research. We
developed a method capable of forming novel gel materials using self-assembling
nanogels as the building blocks (Figure 8.8). Specifically, we found that nanogels
accumulate and form macrogels at a relatively high concentration [99]. We also
developed polymerizable nanogels by synthesizing nanogels containing methacryl-
loyl residues as reaction points [100]. When the nanogels were polymerized with
various hydrophilic monomers, macrogels composed of crosslinked nanogels with
immobilized artificial molecular chaperone functions [101] and quick thermo-
responsiveness [102] were obtained. Macrogels consisting of a network of nanogels
connected by biocompatible polymers can be formed by polymerization between
nanogels and 2-methacryloyloxyethylphosphocholine at relatively high concen-
trations in an aqueous environment.

Recently, macroscopic hydrogels have been used as an artificial extracellular
matrix capable of controlled release of a drug in regenerative medicine. Efficient
tissue regeneration is possible by precisely controlling the release of multiple

1. Design and synthesis of nanogels as building blocks

2. Functional integration of nanogels

3. Advanced biomedical application

Well-organized 3D-structure
Multiple functions
Programmed responses

Tailor-made medicine
Nano-medicine
Regenerative medicine
Cancer vaccine

Figure 8.8 Bionanoengineering fabrication and applications of nanogel-based biomaterials.
cytokines and hormones. Therefore, development of a durable, long-lasting matrix is essential for applications in regenerative medicine. However, unfavorable rapid and bursts of drug release were observed in many cases because of the difficulty in controlling the crosslinking point in the gel, which leads to heterogeneity of the nano-scale structure. Another problem is the denaturation of proteins inside the hydrogels and their loss of function through irreversible aggregation. Biodegradable hybrid hydrogels consisting of acryloyl-substituted CHP and poly(ethylene glycol) (PEG) with four branched terminal thiol groups (CHP–PEG) were used as a matrix for bone regeneration. By allowing the acryloyl group-modified CHP nanogel to absorb prostaglandin E2 (PGE2), a PGE2-encapsulated CHP–PEG hydrogel was prepared [103]. In that study, new bone formation was detected 4 weeks after implantation of the gel into the calvaria of mice. Furthermore, the CHP–PEG hydrogel system exhibited no side effects, such as increases in the cancellous bone of the femur, while bone formation was facilitated specifically at the target site. A CHP–PEG hydrogel encapsulating bone morphogenetic protein-2 within the nanogel markedly facilitated bone formation [104]. It was found that nanoparticles (~150 nm) consisting of several hundred nanogels (~30 nm) crosslinked with PEG-SH were formed when the nanogel-crosslinking reaction was performed at low nanogel concentration [105], a process termed “raspberry-like” nanogel assembly. The resulting raspberry-like nanoparticle was capable of encapsulating interleukin-12 as an immunostimulatory cytokine and was able to keep it even in the presence of BSA in vitro. More importantly, the raspberry-like nanoparticle had a protracted release profile after subcutaneous injection in mice because of hydrolytic degradation under physiological conditions to dissociate back to an original nanogel (Figure 8.9). Therefore, this system enables sustained release of proteins. The simplicity of the preparation and the high encapsulation efficiency will be very advantageous in practical applications.

Figure 8.9  Formation of the raspberry-like assembly of nanogels by crosslinking reactions of PEG-SH. The protracted release profile is due to hydrolytic degradation of the raspberry-like nanogel under physiological conditions, which causes dissociation back to the original nanogel.
8.6 Conclusion and Perspectives

Research in the field of bioengineering on the biomedical applications of nanogels has rapidly progressed over the past 10 years. Their application to the DDS of various drugs has become possible because of the bioengineering techniques that allow us to control the size, stability, and surface properties of nanogels. A future objective of research is the delivery of drugs that are relatively unstable under physiological conditions, particularly proteins, antibodies, and nucleic acids. Nanogels with molecular chaperone functions may offer a promising approach to achieve this goal. Another objective of nanogel research should be the use of nanogels as individual components for building an integrated nanosystem using nanogel engineering. By integrating various functional nanogels using nanogel engineering techniques, the development of nanogel-based biomaterials with a well-organized three-dimensional structure, multiple functions, sensitivity to a range of different stimuli, and programmed responses that can be controlled temporally and spatially may become possible.

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thermosensitive drug release.

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