Science in China Series B: Chemistry

www.scichina.com
chem.scichina.com
www.springerlink.com

Recent developments in intelligent biomedical polymers

XIAO ChunSheng[1,2], TIAN HuaYu[1], ZHUANG XiuLi[1], CHEN XueSi[1†] & JING XiaBin[1]

[1] State Key Laboratory of Polymer Physics and Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, China;
[2] Graduate University of Chinese Academy of Sciences, Beijing 100049, China

Intelligent polymers or stimuli-responsive polymers may exhibit distinct transitions in physical-chemical properties, including conformation, polarity, phase structure and chemical composition in response to changes in environmental stimuli. Due to their unique 'intelligent' characteristics, stimuli-sensitive polymers have found a wide variety of applications in biomedical and nanotechnological fields. This review focuses on the recent developments in biomedical application of intelligent polymer systems, such as intelligent hydrogel systems, intelligent drug delivery systems and intelligent molecular recognition systems. Also, the possible future directions for the application of these intelligent polymer systems in the biomedical field are presented.

stimuli response, intelligent polymers, hydrogels, drug delivery systems, molecular recognition

Living systems consisting of many biopolymers such as proteins, polysaccharides, and nucleic acid regulate their biological functions by precisely responding to the environmental stimuli (e.g. life activities of a single cell). Synthetic polymers that exhibit similar environmental responsive behavior have been extensively investigated for use in intelligent and biomimetic systems. Of particular interest are these intelligent polymers which have found tremendous applications in the biomedical field, including drug delivery, bioseparation, biomolecular diagnostics, biosensor and tissue engineering. Common types of the stimuli-responsive polymers are temperature-responsive, pH-responsive, photo-responsive, electro-responsive, bio-responsive, and multi-responsive polymers. In the present review, intelligent hydrogel systems, intelligent drug delivery systems and intelligent molecular recognition systems based on these intelligent polymers are emphatically introduced. Hydrogel is one of the biomedical materials which have the widest applications. These gel materials are formed from networks of hydrophilic polymers by physical or chemical crosslinking, taking up water whose weight is 10%—20% to several thousand times their own weight. Hydrogels containing stimuli-responsive polymers can undergo volume phase transitions or sol-gel phase transitions in response to external stimuli and thus are used in tissue engineering, biosensor, controlled drug release and so on[1]. For intelligent drug delivery systems, taking polymeric drug delivery nanoparticles (e.g. polymeric micelles or vesicles, etc.) for example, they will undergo deformation/dispersion (micelles, vesicles) or swelling/shrinking (microgels, core/shell cross-linked nanoparticles) in the presence of external stimuli. These properties make it possible for the drug delivery systems to control release at the pathological sites selectively and sustainedly[2,3]. Combining intelligent polymers and

Received May 26, 2008; accepted June 14, 2008
doi: 10.1007/s11426-008-0151-z
†Corresponding author (email: xschen@ciac.jl.cn)
Supported by the National Natural Science Foundation of China (Grant Nos. 20604028, 50573078, and 50733003), National Fund for Distinguished Young Scholar (Grant No. 50425309), A3 Foresight Program of the National Natural Science Foundation of China (Grant No. 20621140369), and Key International Science and Technology Cooperation Project of Ministry of Science and Technology of China (Grant No. 20071314)

bioactive molecules with biorecognition ability, such intelligent biorecognition systems can not only selectively recognize corresponding targets, but also induce dramatic changes in volume, shape, chemical composition or phase structure with external stimuli. Thus, potential applications can be found in bioseparation, biosensor and cancer molecular diagnostics[4].

1 Intelligent hydrogel systems

There are two types of hydrogels, i.e., synthetic hydrogels and natural hydrogels, depending on their origin and composition. Natural hydrogels mostly derive from collagen, hyaluronic acid (HA), fibrin, alginate, agarose and chitosan, while synthetic hydrogels are three-dimensional gels which are swollen but not soluble in water, generated from physical or chemical crosslinks of a series of water soluble polymers such as poly(hydroxyethyl methyacrylate) (PHEMA), poly(vinyl alchol) (PVA), poly(ethylene glycol) (PEG), poly(acrylic acid) (PAA), polyacrylamide (PAAm), and poly(*N*-isopropylacrylamide) (PNIPAM)[1]. Because there are ion-dissociable, polar and hydrophobic groups in the backbones or side chains of the component polymers, the intelligent hydrogels can undergo reversible, discontinuous (or continuous) volume changes induced by the changes of external solvent composition, temperature, pH, electric field, photo, and magnetic field. Thus, it is possible to influence the swelling or shrinking properties through controlling the microstructure and morphology of the polymer gel networks. As a result, hydrogels turn out to be intelligent due to their ability to respond to external stimuli. Depending on the different responsive aspects, intelligent hydrogels can be defined as temperature-responsive, pH-responsive, photo-responsive, pressure-responsive and multi-responsive hydrogels.

1.1 Temperature-responsive hydrogels

All the temperature-responsive polymers have a critical solution temperature. For example, poly(*N*-substituted acrylamide) undergoes phase separation in water when the temperature is higher than its lower critical solution temperature (LCST). Poly(*N*-isopropylacrylamide) (PNIPAM) is one of the most widely studied temperature-responsive polymers. It undergoes sharply reversible phase separation at about 32℃ in water. This property is influenced by its chain structure. There are both hydrophobic group (isopropyl group) and hydrophilic group (amide group) in the side chain of PNIPAM. Therefore, when temperature is below LCST, hydrogen bonds between hydrophilic group and water make the polymers soluble in water. By contrast, as the temperature increases to above LCST, the interactions of the hydrophobic groups are promoted and the hydrogen bonds are weakened, resulting in aggregation of the chains and precipitation of the polymers from water. The LCST of PNIPAM could be adjusted by copolymerization methods, increasing as the NIPAM monomer is copolymerized with hydrophilic monomers and decreasing if hydrophobic monomers are copolymerized into the PNIPAM chains. PNIPAM hydrogels are typical thermo-shrinking hydrogels, which deswell above LCST and swell below LCST. Although the temperature sensitivity of PNIPAM is great, the PNIPAM hydrogels require even several weeks to reach equilibrium, which brings about hindrances to the applications of such hydrogels. In order to solve this problem, several strategies have been investigated. Yoshida et al.[6] synthesized a comb-type grafted hydrogel (PNIPAM grafted PNIPAM) with fast deswelling properties. When the temperature increased, the free grafting side chains generated some hydrophobic cores due to the hydrophobic interactions, which dramatically enhanced the aggregation of the crosslinking points, resulting in decrease of the deswelling time from more than one month to about 20 min (Figure 1). More recently, comb-type grafted polymer hydrogel with amphiphilic polymers as the grafting chains was synthesized by Xu et al.[7]. The swelling ratio of this hydrogel was higher than that of conventional ones and its deswellling rate was also fast.

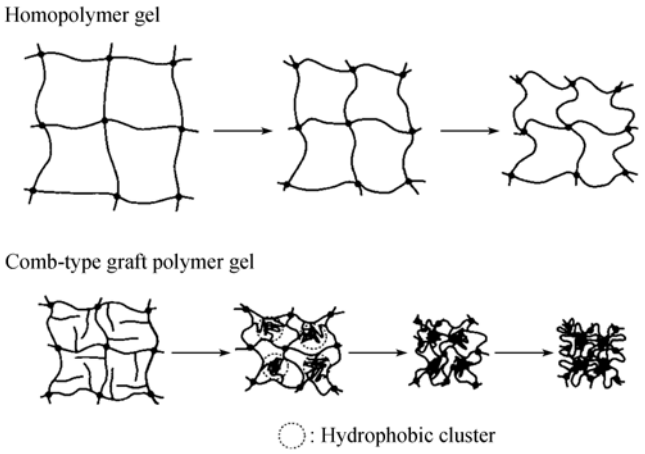

Figure 1 Structure and deswelling mechanism of homo- and comb-type grafted PNIPAM hydrogel[6].

Besides being insensitive, poor mechanical property is another disadvantage of the conventional PNIPAM hydrogels. One of the effective methods to improve the mechanical property of hydrogels is to form the interpenetrating polymer networks (IPN). IPN is a kind of unique polymer blend or polymer alloys structure, generated from interpenetrating entanglement networks. The special force within the IPN is able to combine two component polymers with great differences or diverse functions stably so as to realize complementation among different components. Simultaneously, the cellular structure, interface interpenetration and bicontinuous structure of IPN enable the synergy of their properties or functions, leading to enhanced mechanical property and improved responsiveness of the hydrogels. In a recent study, PNIPAM hydrogels containing silk fibroin interpenetrating networks were synthesized[8]. The results showed that the presence of silk fibroin with β-sheet structure could increase not only the viscoelasticity but also the deswelling rate. Another strategy to improve the mechanical property is to synthesize organic/inorganic hybrid hydrogels. Haraguchi et al.[9] have synthesized PNIPAM/clay nanocomposite hydrogels exhibiting good mechanical property superior to common PNIPAM hydrogels. Moreover, the hybrid hydrogels could withstand large amplitude stretching, bending and compression without destruction, and even could be stretched up 10 times or knotted.

Polymer gels based on hydrophilic-hydrophobic balance are another type of temperature-responsive hydrogels. They undergo reversible sol-gel transitions in aqueous solution via changing the hydrophobicity of the physical crosslinking area in response to temperature stimuli. Therefore, these hydrogels different from those undergoing volume phase transitions are always used as injectable hydrogels applied in non-invasive or minimally invasive treatments.

Poloxamers® and Pluronics® are general designation of a kind of nonionic surfactant. They are a series of amphiphilic ABA triblock copolymers with poly (ethylene oxide) (PEO) as the A segment and poly(propylene oxide) (PPO) as the B segment. A general mechanism of the thermo-gelling behavior has been proposed that copolymers firstly form micelles in the aqueous medium as the temperature increases, and then interpenetrations between micelles leading to gelation as the temperature further increases[10]. However, applications of these hydrogels encounter several limitations. Firstly, a relatively high concentration is required to form hydrogels at body temperature, which is a hindrance to apply such hydrogels *in vivo*. Then, poly(1,2-butylene oxide) (PBO) as a more hydrophobic segment is introduced to replace the PPO block, causing gelation of the copolymers solution at a lower concentration. Likewise, biodegradable polyesters (i.e., poly(lactic acid), poly(glycolic acid), poly(ε-caprolactone) and their copolymers) can be also used to copolymerize with PEO block in place of PPO. The resultant copolymers not only have good biocompatibility and biodegradability, but also settle the problem that poloxamer hydrogels are erosible from its surface into soluble unimers quickly *in vivo*[5]. In a case study, Zentner et al.[11] synthesized low molecule weight poly (lactide-co-glycolide)-poly(ethylene glycol)-poly (lactide-co-glycolide) (PLGA-PEG-PLGA) for delivery of proteins and water-insoluable drugs. Biodegradable experiments showed that hydrogels were sorbed almost completely over 4 to 6 weeks after being injected into rats. *In vitro* studies indicated that the release of Paclitaxel from hydrogels sustained up to 50 days, however the Poloxamer 407 released the paclitaxel within 1 day.

When the temperature decreases, most of the naturally derived thermosensitive polymers undergo gelation in aqueous solution, such as gelatin and carrageenan. They adopt random coil conformation at higher temperatures, and transform it to helix by lowering the temperature, during which physical crosslinking networks occur. Some derivatives of cellulose present an opposite mechanism of gelation. They are soluble in water at a certain concentration at lower temperatures, while forming gel networks at higher temperatures. Tate et al.[12] have investigated methylcellulose-based polymers as the tissue engineering scaffold for repairing traumatic brain injury. These polymers show low viscosity in aqueous solution below 23℃ and gelation occurs in brain at body temperature. The biocompatibility of the hydrogel was assayed to be good both *in vitro* and *in vivo*. Another example is that ethyl(hydroxyethyl) cellulose and surfactant complexes used as a drug delivery system for peridental anesthesia were studied by Scherlund et al.[13]. Results showed that the gelation behavior would not be disturbed by addition of the local anesthetic agents, lidocaine and prilocaine. And the sustained release time achieved from the complex gels was at least 60 min, which may be used as short-term drug sustained

release systems. However, the presence of poisonous ionic surfactants in such system has limitations on its applications. Chitosan/glycerol phosphate is another polysaccharide-based reversible gel system. Chitosan is a kind of biocompatible cationic polymer which is soluble in acid medium and forms gelatinous precipitate as the pH increases, while forming thermo-sensitive hydrogels in the presence of polyol salts (such as glycerol phosphate). Hoemann et al.[14] reported some experiments on the proliferation of primary bovine chondrocytes on chitosan/glycerol phosphate hydrogels. Mechanical strength test revealed the deposition of functional matrix after being cultured *in vitro* for 3 weeks. It was also found that chitosan/glycerol phosphate hydrogels could adhere well to around the bone and cartilage after injection of its aqueous solution into rabbit with bone defects. Subsequently, they used chitosan/glycerol phosphate/blood complex system to repair cartilage. Such complex systems were named CarGel® and used *in vivo* experiments on rabbit, sheep and horse, indicating that there were no local or systemic side effects.

1.2 pH-responsive hydrogels

The pH-responsive hydrogels are another kind of widely studied stimuli-responsive hydrogels. Its swelling/deswelling behavior occurs in response to the environmental pH changes. In general, there are plenty of ionizable groups in the macromolecular networks of pH-responsive hydrogels. As the variation of environmental pH, these groups are ionized to cause differences of internal and external ionic strength and breakage of hydrogen bonds between the molecular segments. As a result, the decrease of crosslink densities and the increase of electrostatic repulsions lead to macroscopical swelling of the hydrogels. According to the different functional groups, there are three types of pH-responsive hydrogels defined as anionic, cationic and zwitterionic hydrogels. Anionic pH-responsive hydrogels generally have $-COO^-$, and $-OPO_3^{2-}$ as its sensitive pendants. The most commonly used monomers are acrylic acid (AAc) and its derivatives. Cationic pH-responsive hydrogels generally have basic pendants such as primary amine, secondary amine and tertiary amine. The most commonly used monomers are *N*,*N*′-dimethyl aminoethyl methacrylate, 2-vinylpyridine, etc. Zewitterionic pH-responsive hydrogels consist of both acid and basic ionizable groups, obtained by crosslinking of different polyions.

Kadlubowski et al.[15] synthesized hydrogels consisting of poly(vinyl pyrrolidone) (PVP) and poly(acrylic acid) (PAAc) via photo-initiated cross-linking. These hydrogels collapsed, turned to turbidity and exhibited phase separation at low pH due to the protonation of carboxyl groups. These properties are absent in the hydrogels that only contain PAAc. Another kind of hydrogel based on semi-IPN of chitosan (CS) and PEO were synthesized and studied in comparison with crosslinked CS hydrogels[16]. Results showed that the swelling of the former was highly dependent on pH, and the swelling and mechanical properties were all superior to the latter. Brahim et al.[17] synthesized hydrogels by copolymerization of 2-hydroxylethyl methacrylate (HEMA), 3-(trimethoxylsilyl)propyl methacrylate (PMA) and *N*,*N*′-dimethyl aminoethyl methacrylate (DMAEMA), by using tetraethylene glycol diacrylate (TEGDA) as a cross-linker. In the presence of PMA which could crosslink with hydroxyl groups of HEMA, the maximum swelling ratio decreased with the increase of the mole ratio of PMA in monomers. These hydrogels to swell at low pH are considered to have potential applications in pH-responsive delivery of hydrophobic drugs.

Anionic hydrogels that shrink in acid medium and swell in neutral or basic medium have been investigated, proving that they can be used in oral or colonic targeting delivery of peptide or protein drugs. Because the shrinkage in acid medium can protect the peptide or protein drugs from being destroyed in stomach, the swelling and bio-adhesion in colonic neutral or slightly basic environment may be favorable to drugs release and absorption. Park et al.[18] synthesized pH-responsive hydrogels using poly(vinyl alcohol) (PVA) as the networks backbone grafted with PAAc and poly(methacrylic aicd) (PMAAc). *In vitro* studies on insulin release showed that there was no visible release of insulin in simulated gastric fluid (pH 1.2), but controlled release of insulin was observed in simulated intestinal fluid (pH 6). Further studies on the oral delivery behavior *in vivo* in a rat model confirmed that the effectively released insulin could control the level of glucose within 4 h. Murthy et al.[19] synthesized acid-labile acetal crosslinked hydrogels and microgels. Release behavior was studied using bovine serum albumin (BSA) as a model drug, showing that at pH 7.4, the release rate was so slow that only 5% BSA was released after 2 h and a relatively

long time (96 h) was required to obtain complete release. By contrast, under an acidic condition (pH 5.0), the crosslinking points were destroyed quickly through acidolysis, leading to fast release of loaded BSA within 2 h. Moreover, gel electrophoresis confirmed the retained bioactivity of the released BSA.

1.3 Electro-responsive hydrogels

Electro-responsive hydrogels are a kind of intelligent hydrogels to undergo swelling, shrinking and bending behavior in response to electro stimuli. One of its unique characters is that such hydrogels can transform the electric energy to mechanical energy. Electro-responsive hydrogels generally consist of polyelectrolyte. Their responsive property is attributed to the directional migration of free ions in solution in DC electric field. The migration of free ions within hrdrogel causes inhomogeneity inside and outside the hydrogel, leading to variation of osmotic pressure and deformation of hydrogel. Therefore, these hydrogels with ability to transform electric energy to mechanical energy have been investigated significantly as energy converter applied in fields such as robots, sensors, controlled drug release and artificial muscles.

These hydrogels have been investigated since 1965. Hamlan et al.[20] firstly reported that the ionic PVA hydrogels connected to anode shrank in 1% NaCl solution after applying 5V DC voltage. Subsequently, Tanaka et al.[21] found that partially hydrolyzed polyacrylamide hydrogels in water-acetone underwent discontinuous volume changes in the presence of electric field, and recovered to its original form as the electric field was removed. Because of the poor mechanical property of hydrogels made by simplex polymerization, copolymerization or blends are adopted to obtain electric hydrogels with better mechanical strength. Yao et al.[22] synthesized crosslinked hydrogels bearing sulfonic groups via sulphonation of polystyrene-polyethylene-polybutylene block copolymers. These hydrogels bent towards cathode in electric field after swelling in saline solution, but bent towards anode after swelling in deionized water. Semi-IPN hydrogels consisting of polyaniline and chitosan were prepared by Kim et al.[23]. The swelling ratio and electric sensitivity were studied at various pH. A relatively high swelling ratio was observed at low pH. And the gel membranes showed diverse mechanical behavior depending on the pH in DC electric field. It bent to anode in buffer solutions at pH of 1.0 or 4.0, while bending to cathode at pH of 10.0. This is attributed to the fact that the polyaniline has different electro-activity under these conditions. An oscillatory bending was observed in neutral (pH 7.0) buffer solution, which might be attributed to reverse transformation between the electro-activity state and the oxidation state of polyaniline.

1.4 Photo-responsive hydrogels

The responsive property of the photo-responsive hydrogels is mainly based on the following three mechanisms: (1) Photodegradable compounds are conjugated to the polymer gel and generate a number of ions within gel when exposed to light. The marked increase of ions induces abruptly changes of the osmotic pressure within gel which allows the solvent outside to diffuse towards inner part and volume phase transition occurs subsequently in a manner of photo-response. (2) Introducing photosensitive compounds into a temperature-sensitive hydrogel, local temperature within the hydrogel will increase when the photosensitive compounds absorb a number of protons and convert them to heat. Volume phase transition occurs if the temperature continues increasing up to the phase transition temperature of the hydrogel. (3) Photosensitive groups to undergo electronic transitions from ground state to excited state by absorbing a certain amount of photon energy are introduced into backbone or side groups of polymers. The molecules in excited state undergo isomerisation through intramolecular or intermolecular energy transfer which induces changes in molecular conformations, and further results in physical-chemical changes within hydrogel. Thus, these hydrogels make response to photo-stimuli corresponding to the isomerization of the photosensitive groups. For instance, azobenzene and its derivatives can undergo *cis-trans* isomerisation under UV illumination, which may change the molecular sizes and the distance between macromolecules, resulting in volume changes of the gel.

Desponds et al.[24,25] prepared a photosensitive copolymer based on copolymerization of NIPAM and *N*-acryloxysuccinimide (NAS), and subsequently conjugated chromophore group, 3-aminopropyloxy azobenzene, to the side chain of NAS. The LCST of the resultant polymer was 16℃ in stable *trans* form of azo group. If exposed to 330 nm UV illumination, the azo moieties converted to more hydrophilic *cis* form, and the LCST increased to 18℃. However, the azo groups could

be recovered to *trans* form under 440 nm visible light illumination. Thus, the photo-modulated temperature-sensitive point can be achieved. Zheng et al.[26] reported that the sol-gel transition point of the azobenzene-functionalized hydroxypropyl methylcellulose would shift to high temperatures if exposed to UV illumination, but shift to low temperatures, by contrast, if adding a small amount of α-cyclodextrin. This is because the α-cyclodextrin could interact with *cis* zaobenzene groups under the illumination of UV. Therefore, the different shifting trends of the sol-gel transition point in the presence of α-cyclodextrin have opened a new avenue to investigate hydrogels with photoregulated temperature-sensitive point.

1.5 Pressure-responsive hydrogels

Pressure-responsive hydrogels are a kind of hydrogels that undergo volume phase transitions in response to the changes of external pressure. The pressure dependence of hydrogels was firstly proposed by Marchetti[27] through theoretical calculation. The calculated results suggested that hydrogels collapsed at a low pressure, while expanding at a high pressure. This prediction was confirmed by experiments conducted by Lee et al.[28]. They prepared PNIPAM hydrogels using *N*,*N*′-methylenebisacrylamide as a cross-linker. Results showed that the volume of hydrogels changed in response to pressure variation and suggested that the pressure-dependent volume changes of hydrogels might be attributed to the contribution of pressure to the systemic free energy.

1.6 Multi-responsive hydrogels

Hydrogels with a single sensitivity usually can not satisfy the actual application demands, especially the applications in complex environment and multi-intelligent systems. Accordingly, multi-responsive hydrogels with ability to respond to different external stimuli would be more available. For example, Shim et al.[29] synthesized an injectable temperature and pH double sensitive hydrogel. Sulfamethazine oligomers serving as pH sensitive components were coupled to the ends of temperature-sensitive poly(ε-caprolactone-*co*-lactide)-poly(ethylene glycol)-poly(ε-caprolactone-*co*-lactide) (PCLA-PEG-PCLA), leading to a temperature and pH sensitivity system. This copolymer could undergo a sol-gel transition within a narrow range around the physiological pH. It formed a gel at pH 7.4 and 37℃, but presented as a sol at pH 8.0 and room temperature. Bhattacharya et al.[30] synthesized a temperature, pH and magneto- sensitive hybrid microgel. This microgel was prepared by addition of magnetic particles while preparing temperature and pH sensitive microgel. The content of magnetic particles could be adjusted by the concentration of ferric salt (maximum up to 15%) without influence on the stability and other two kinds of sensitivity of the microgel.

2 Intelligent drug delivery systems

Before 1970s, most of the drug administrations are traditional tablets, drops and injections. But no matter whether drugs act on an organ or system, most of them have a therapeutic window, that is, drugs should generate an effective concentration in the pathological area. This leads to new requirements on the design of drug delivery systems that could control concentration of drugs at the targeted sites. Meanwhile, some drugs exert their effects not only at the targeted sites but also at the adjacent sites and cause side effects, which demand to control the drugs distribution after entering humoral system and tissues. To meet the new demands of drug administrations, intelligent drug delivery systems have been developed to release drugs timely, quantitatively and site-selectively in response to the variation of pathological signals such as pH, temperature, or biomolecules in order to gain a better efficacy.

2.1 pH-Responsive drug delivery systems

Drugs will encounter complex environmental pH inside the body. For example, oral administration will go through acidic environment in stomach and then neutral or slightly basic intestine, while anti-caner drugs will come up against lower pH values in tumor and inflammatory tissue (pH ~6.8－7.2) as well as in endosome and lysosome of cells (pH ~5－6) compared with pH ~7.4 in normal tissue and blood. Therefore, pH-responsive drug delivery systems have been developed mainly based on the following two types: (1) Nanoparticles with basic groups (e.g. amines) in the core are protonated in acidic solution and the hydrophobic core is changed into a hydrophilic system, leading to dissociation or swelling of particles and release of the loaded drugs, which is suitable for controlled drug delivery in tissues and cancer cells. (2) By contrast, nanoparticles with acidic groups (e.g. carboxyl) in the core become hydrophilic by deprotonation in basic solution and can be used as controlled drug delivery in intestine[3,5].

More examples have been focused on the first type of drug delivery system. This kind of system is constructed by polymers with basic groups which show hydrophobicity at a pH above its pK_a and form stable drug-loaded particles by conjugating hydrophilic polymer segments. As the pH decreases below the pK_a, the protonation of polymers turns the "hydrophobic segments" to hydrophilic ones and increases the electrostatic repulsion between charged groups. For example, micelles consisting of poly(L-histidine) and PEG (pHis-PEG) dissolved at physiological pH 7.4. But micelles constructed by PLA-PEG and pHis-PEG were stable at pH 7.4 and dissolved at pH 7.2. This dissociation pH depends on the blending ratio of PLA-*b*-PEG and pHis-PEG[31,32]. Block copolymer poly(2-vinylpyridine-*b*-ethylene glycol) (P2VP-PEG) formed stable vesicles in neutral and alkaline solution. However, these vesicles dissolved at pH 5 and fast released loaded fluorescent dye at pH 4, which showed potential applications in pH-responsive drug delivery systems[33]. Irvine et al.[34] reported a kind of crosslinked core-shell nanoparticle using pH sensitive poly(*N*,*N*′-diethyl amioethyl methacrylate) (PDEAEMA) as the core and poly(2-aminoethyl methacrylate) (PAEMA) as the shell, which could effectively deliver the membrane-impermeable molecules, such as calcein and ovalbumin, into cytosol of dendritic cells. It was showed that these nanoparticles could effectively disrupt endosomes due to their pH-responsive core and then released the loaded drugs into cytosol without overt cytotoxicity.

The type (2) drug delivery systems show an opposite pH sensitivity. These systems remain stable at low pH while releasing drugs at high pH, which can usually be used for oral administration. For example, a series of pH-responsive block copolymers were developed by Leroux et al.[35,36] by using PEG as the hydrophilic component and poly(alkyl acrylate-*co*-methacrylic acid) as the pH sensitive core. Depending on the polymer composition, these block copolymers formed micelles with the sizes from 120 nm to 350 nm at pH < 4.5, while releasing drugs as the pH increased up to 7.2. Another study performed by Sarmento et al[37]. was that dextran slufate/chitosan nanoparticles were investigated for oral delivery of insulin. Insulin can be fully retained by dextran sulfate at low pH in stomach and released in neutral intestine. The mucoadhensive property of polysaccharide nanoparticles also enhanced the uptake of insulin via intestinal mucosa. The insulin association efficiency was over 70% and the release of insulin was in a pH-dependent manner under simulated gastrointestinal conditions. A significant increase of efficiency over 1.6% in comparison with direct administration was observed in treatments of diabetic rats.

In recent years, pH-induced charge-reversal polymeric nanoparticles have received great attention to applications in drug delivery systems. Lee et al.[38] firstly synthesized PEG and poly(β-benzyl-L-aspartate) block copolymer and then modified it into PEG-poly ((2-aminoehtyl)aspartamide) bearing positive pendant groups by aminolysis with excess ethylenediamine. This copolymer further reacted with citraconic anhydride to transform the positive pendant groups into negative carboxyl and meanwhile had acidic degradable citraconic amides in the pendants. These polymers were used to form polyion complex (PIC) micelles with cationic model protein. The PIC micelles disintegrated and selectively released the active protein owing to charge-conversion of the pendants caused by the acidolysis of citraconic amides. Therefore, these pH-responsive block copolymers are promising for controlled protein drugs delivery. Similarly, Xu et al.[39] synthesized PCL and polyethyleneimine (PEI) block copolymers (PCL-*b*-PEI). The primary and secondary amines of PEI block were partially converted into amides to form micelles with negative surface by reacting with *cis*-1,2-cyclohexanedicarboxylic anhydride. At low pH, the hydrolysis of amides led to recovery of the micelles surface into positive charge (Figure 2). *In vitro* experiments revealed that the doxorubicin (DOX)-loaded nanoparticles could effectively kill SKOV-3 ovarian cancer cells and its negative surface at neutral pH could reduce interactions with proteins in blood, which prolonged the circulation time with enhanced efficiency. Therefore, these interestingly designed nanoparticles may have potential applications in drug delivery *in vivo*.

2.2 Temperature-responsive drug delivery systems

Poly(*N*-isopropylacrylamide) (PNIPAM) is considered to be the most commonly studied temperature sensitive polymer. When PNIPAM block is copolymerized with hydrophobic segment, it forms micelles in neutral aqueous solution with hydrophobic segment as the core and PNIPAM segment as the shell. This type of micelles presents typical temperature sensitivity. The hydrophilic

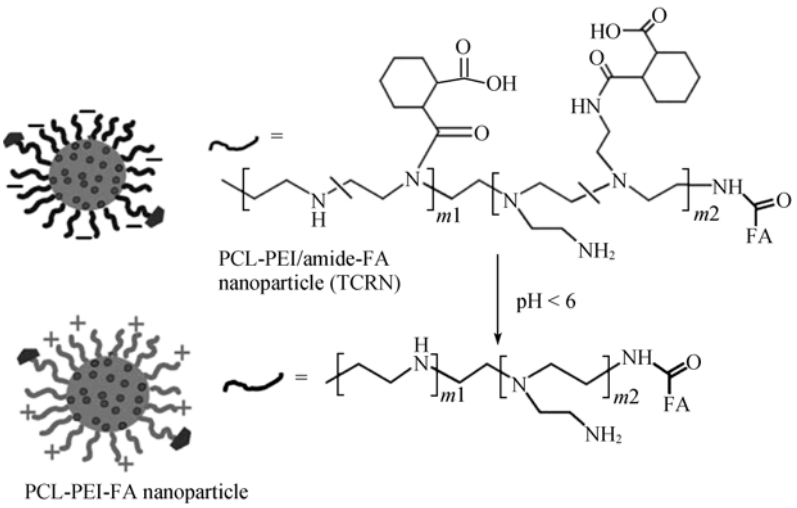

Figure 2 The structure of folate-targeted charge-reversal nanoparticles and its pH-induced charge reversal[39].

PNIPAM turns hydrophobic at the temperature above LCST which induces dissociation of micelles, while as the temperature decreases below LCST, PNIPAM reverts to hydrophilicity and micelles are reformed. This course is reversible in aqueous solution. Drug delivery systems using such kind of copolymers are feasible to control drug release by facile healing/cooling steps to adjust the structure of micelles. Yang et al.[40] synthesized a series of block copolymers based on poly(*N*-isopropylacrylamide-*co*-*N*,*N*′-dimethylacrylamide) (P(NIPAM-*co*-DMAAm)) and poly(lactide-*co*-glycolide) (PLGA). These copolymers formed micelles in phosphate buffer solution (PBS) and their LCST was all around 39℃. The micelles loaded with DOX were stable and released DOX slowly in serum-contained PBS at 37℃, while they were destroyed and released DOX quickly as the temperature rose to 39.5℃. This kind of micelles with temperature-dependent structure reversal property provides an intelligent drug delivery system for local hyperthermia.

However, drug delivery systems with simplex temperature-responsive character usually can not meet the clinical requirements. It should be used in combination with pH or photo responsive component. For example, Sershen et al.[41] developed a photothermal responsive nanoshell-composite hydrogel using thermal responsive poly(*N*-isopropylacrylamide-*co*-acrylamide) in combination with photo sensitive gold nanoshell. The gold nanoshell could absorb near IR irradiation (1604 nm) and convert it to heat leading to collapse of hydrogel and release of the loaded drugs. Additionally, using BSA as the model protein, pulsatic release behavior could also be achieved by modulating the on-off near IR laser irradiation.

2.3 Glucose-responsive drug delivery systems

Diabetes presently is one of the most prevalent diseases in modern society. It is caused by the deficiency of insulin which can not control the glucose concentration in blood. To treat diabetes, the most frequently used method is that a predetermined amount of insulin is introduced to control the metabolism of blood glucose according to the monitoring concentration of blood glucose. But this method is too complicated with its efficiency being not satisfactory. Thus, many efforts have been made attempting to develop intelligent drug delivery systems. These systems themselves may control the release rate and amount of insulin depending on the concentration of blood glucose, i.e., the so-called glucose-responsive drug delivery systems.

One of the most commonly used glucose-responsive drug delivery systems is to combinate glucose oxidase into pH sensitive hydrogels, membranes or capsules. Their insulin release behavior is based on the following mechanism: when glucose enters the insulin-loaded system, the glucose oxidase converts glucose to gluconic acid which decreases the microenvironmental pH resulting in swelling of the system and release of insulin[42].

Park et al.[43] prepared glucose sensitive hydrogels by blending the glucose bearing polymers and PEGlated Con A. Studies on the modulated release of insulin from these hydrogels showed that the release rate depended

on the concentration of glucose. The release rate increased as the glucose concentration increased from 1 to 4 mg/mL, but decreased as the glucose concentration decreased to below 1 mg/mL.

2.4 Antigen-responsive drug delivery systems

For treatment of infectious disease, cancer and hypersensitivity, the ultimate efficacy relies on directly induced proper immune response. With the improvement of biotechnologies, researches on the delivery systems applied to low molecular weight proteins, peptides and DNA have become more and more important. And most of the researches on the controlled delivery systems have been laid on antigen and hypersensitive drugs.

Depending on the characters, antigen can be classified into two types. The one with both immunogenicity and immune reactivity is called complete antigen, such as bacteria, viruses and serums from heterogenous animals, the other that only has immune reactivity with corresponding antibody or sensitized lymphocytes but no immunogenicity is called hapten, such as most polyssacharides, lipid and some simple chemicals. They do not have immunogenicity themselves but obtain it after forming complexes with protein macromolecules. This kind of protein macromolecule to form complexes with antigens and endow the complexes with immunogenicity is called carrier.

Metastatic renal cell carcinoma (RCC) is an aggressive disease refractory to most existing therapeutic modalities. Identifying new markers for disease progression and drug targets for RCC will benefit this unmet medical need. Wahlaf et al.[44] used the anti-CD70 as a drug carrier that targeted to CD70 whose expression was found to maintain at the metastatic sites of RCC. Hence, these anti-CD70 antibody-drug conjugates (ADCs) could selectively recognize RCC, internalize, and reach the appropriate subcellular compartments for drug release and tumor cell killing.

2.5 Targeted drug delivery systems

Majority of the therapeutical drugs have side effects. Especially, the anticancer drugs kill the cancer cells while inducing death of normal cells. Therefore, intelligent drug delivery systems are required not only to control release according to biological microenvironment, but also to selectively enter the pathological tissue or cells after being introduced into body. Compared with naked drugs to distribute nonspecifically in organs and tissues after administration, nanoparticle drug delivery systems can selectively accumulate in pathological tissue. This phenomenon is called EPR (enhanced permeability and retention) effect[45,46]. The mechanism is that the rapid and abnormal angiogenesis in tumor tissue enhances the permeability of the blood vessels in tumors, which facilitates the leakage of nanoparticles into tumor interstitial space; on the other hand, the immune system with poor lymphatic drainage of the metabolic products in tumors makes it possible to retain the nanoparticles in the tumors, leading to the targeted release of the loaded drugs. Researches using liposomes with different mean sizes suggested that the suitable vesicle size for accessing tumors was about 400 nm, but other studies showed that particles with size < 200 nm were more effective [47]. Although passive targeting approaches based on EPR effect have received more attention in fundamental and clinic investigation, they suffer from several limitations. For instance, the distribution of nanoparticles in tumor tissue is lack of control, leading to over accumulation of the drugs in partial areas which may induce resistance of cancer cells towards a variety of drugs (multiple-drug resistance, MDR). Moreover, the EPR effect is absent in some tumors and the permeability of vessels may be not the same even within the same tumor tissue[48].

However, active targeting has no such limitations as described above. The active targeting drug delivery systems are able to selectively deliver drugs to targeted cells achieved by conjugating targeting agents (e.g. some biomolecules that can recognize and bind specific receptors on cell surface) to the surface of nanocarriers. In general, the agents used for targeting delivery are imperative to bind selectively the specific receptors of the cell surface. Based on the present studies, targeting agents general can be classified into the following types: (1) antibodies and their fragments; (2) small proteins and antibody analogues: fusion proteins, avimers, affibodies, etc.; (3) aptarmers: short single-stranded DNA or RNA; (4) other receptor binding ligands: growth factor, folate, transferrin, RGD peptide, etc.[48,49]. For example, in order to deliver specifically anti-arthritic drug, indomethacin, to inflammatory regions and investigate its biodistribution in the vicinity areas, a number of folate-dendrimer conjugates were prepared by Diwan et al.[50] and applied to the delivery of anti-anthritic drug to inflammatory tissues. These systems used G4-PAMAM as the main body conjugated with folate by CDI chemistry on the surface, and then indomethacin was loaded. The *in vitro* release rate decreased for the folate-conju-

gated dendrimers when compared with unconjugated ones. However, the half-life and residence time of indomethacin in inflamed region were longer for folate-dendrimer conjugates. In addition, the time of the drug exposed in inflamed area and targeting efficiency were all superior to unconjugated dendrimers, suggesting that the folate-targeted drug carrier has potential applications in targeted delivery of anti-arthritic drugs.

3 Intelligent recognition systems

Molecular recognition is ubiquitous and essential in natural life processes, such as enzyme/substrate binding, antigen/antibody interactions, receptor/ligand interactions, complementary RNA or DNA hybridizations. Polymers with recognition ability can mimic the life processes, recognizing specific targets based on the designs of polymer composites and architectures. Thus, these polymers were found to have wide variety of applications in drug delivery, molecular diagnostics and bioseparation[4,51−54].

One of the most direct strategies to prepare polymers with recognition ability is to combine functional intelligent polymers with bioactive molecules through conjugation chemistry, leading to bioconjugate/hybrid systems. Bioactive molecules used in bioconjugations include proteins, peptides, sugars, polysaccharides, single-/double-stranded oligonucleotides, DNA plasmids, simple lipids and phospholipids, a wide spectrum of recognition ligands and synthetic drug molecules[4]. Additionally, the functional intelligent polymers are externally stimuli-responsive (such as temperature-, pH- and photo-responsive). The combination of bioactive molecules and intelligent polymers building up "double smart" systems, and integrating properties of the two components, may have attractive potential applications in biomedical fields.

Bioaffinity separation is an important method to separate and purify bioactive molecules, such as proteins and nucleotides. Bioactive molecules randomly conjugate to the polymer pendant groups are allowed to bind targeting molecules specifically, afterwards, external stimulus is applied to inducing phase transition of polymer. As a result, phase separation of the system and the separation and purification of the targeting molecules are achieved. At present, affinity precipitation systems based on such mechanism as described above have been applied in separation and purification of proteins and nucleotides. Figure 3 shows a typical bioconjugate which contains temperature sensitive poly(*N*-isopropylacrylamide) (PNIPAM)[4]. After the LCST polymer-ligand conjugates bind corresponding affinity receptor, raising temperature causes precipitation of affinity complex from aqueous medium. And then the purified affinity receptor can be recovered by eluting the complex. Another advantage of this bioconjugate is that the LCST polymer-ligand conjugates could be recycled. Therefore, affinity precipitation separation used as a simple and economical purification method for bioactive molecules has received more and more attention. For example, Hoffman et al.[4,53] used PNIPAM-protein conjugates to recover the enzyme from its reaction solutions and applied them to affinity precipitation separation of IgG. Similarly, Maeda et al.[54] synthesized a vinyl derivative of a single-stranded DNA $(dT)_8$ and used it to copolymerize NIPAM monomers in pH 7.4 buffer solution to yield PNIPAM-$(dT)_8$ copolymer. The results showed that the conjugate copolymer could effectively precipitate $(dT)_8$ complementary single-stranded DNA $(dA)_8$ at a rate of 84%, while only a small amount of $(dA)_8$ (6%) was precipitated in the absence of the conjugate copolymer. The applicability of the affinity precipitate system was further examined in a mixture of $(dA)_8$ and $(dA)_3dT(dA)_4$. The results indicated that PNIPAM-$(dT)_8$ copolymer could selectively precipitate the complementary $(dA)_8$ from aqueous solution other than the $(dA)_3dT(dA)_4$. Moreover, the affinity phase separation concept can be further extended to selective isolation and assay of analytes from more complex systems, such as serum. In this case, another labeled antibody was introduced to bind targeting antigen, and then formed a ternary sandwich complex consisting of polymer-antibody conjugate, targeting antigen and labeled

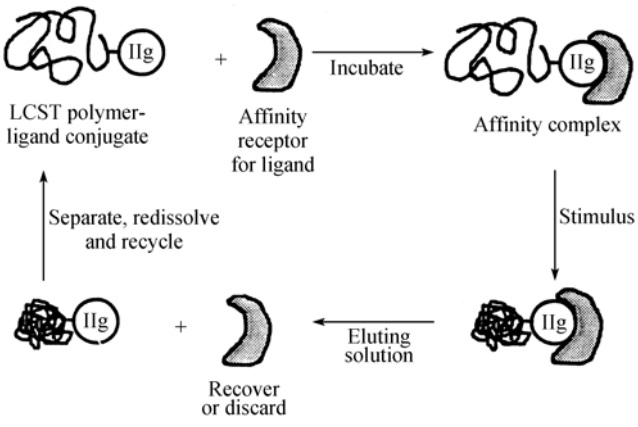

Figure 3 Schematic of affinity precipitation separation based on molecular recognition[4].

antibody. The complex could undergo phase separation under the external stimuli, resulting in the isolation and assay of the targeting analytes. Thus, this method was expected to be used as enzyme linked immunosorbent assay (ELISA) in solution[4,53].

However, these random conjugations have a significant disadvantage, especially for proteins. The space hindrance and the conformation of proteins may be changed after conjugating polymers, leading to decreasing and even losing the bioactivity of protein[4,55]. Therefore, preparation of polymer-protein conjugations with well-defined structures is the precondition of their application in biomedical fields. One strategy is to introduce cysteine residue to a specific site in protein by gene engineering and then use it to conjugate polymers. The site specific polymer-protein conjugations could not only retain the bioactivity of the proteins, but also control the bioactivity through controlling the hindrance at the recognition site[56]. Moreover, recent developments in controlled polymerization also facilitate the synthesis of well defined polymer-protein conjugates. Controlled living radical polymerization methods such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer polymerization (RAFT) can be used to synthesize well defined polymer-protein conjugates in one-pot by coupling initiators to protein and subsequently using the protein macromolecular initiators to initiate polymerization directly in PBS at mild conditions[57−59]. It is believed that more and more polymer-protein conjugates with unique properties may be synthesized by gene engineering technology and controlled polymerization to gain new applications.

As the development of glycochemistry and glycobiology (i.e. glycomics), it has been more and more recognized that sugar plays a key role in biological process including inflammation, cell-cell interactions and signal transmission[60,61]. However, the difficulties in synthesis and isolation of carbohydrates have greatly limited its applications in carbohydrates molecular biology research and clinical pathogen detection, anti-infection drugs and vaccine development[60]. Homopolymers bearing carbohydrates pendants and its copolymers combining with stimuli-responsive polymers (i.e. glycopolymers) obtained by polymerization strategies are considered to be promising in mimicking the function of oligosaccharides and polysaccharides and have been investigated in diverse applications including glcomics, macromolecular drugs, molecular diagnostics and bioseparation[61−63]. It has been reported that glycopolymers that agglutinated bacteria through interactions with receptors on the surface of bacteria could be used to detect and deactivate pathogens[64−66]. Recently, Alexander et al.[63] prepared two sets of glycopolymers with LCST at 41℃ and 44℃, respectively. The glycopolymers were synthesized by using thermo-responsive PNIPAM as the central component and incorporating glucose to the side chains. At room temperature, the interactions between the sugar moieties on the polymers and the cell-surface receptors (*fim* H protein) resulted in aggregation of the *E. coli*. As the temperature increased, glycopolymers underwent coil-globules transition and then no aggregation of the *E. coli* was observed due to the unavailable glucose residues in the globules state for interaction with *fim* H protein. This reversible thermo-control of bacteria aggregation is promising for application in separation and detection of pathogens. Miura et al.[62] also reported a series of glycopolymers prepared by direct polymerizing of vinyl saccharide and used them to investigate the recognition ability and interactions with protein receptors. The results showed that the glycopolymers could recognize the protein receptors specifically with a binding constant being more than 10 times the monomeric oligosaccharides. More interestingly, glycopolymers also have affinities with pathogens of protein toxin, bacteria and virus, and subsequently inhibit their bioactivities. This strategy may find potential applications in development of toxin inhibitors and anti-infection drugs.

Molecular imprinting technology is a rapidly developed and effective method to create artificial recognition sites in the polymer matrix. It is produced by mixing functional monomers, cross-linkers and templets in advance followed by polymerizing *in situ* to form crosslinked networks. Cavities with recognition ability were formed after removal of the templets. The resultant molecular imprinting polymers (MIP) have an affinity constant comparable to natural products and can recognize different targets according to the selections of different monomers, cross-linkers and templets. Also, the molecular imprinting polymers capable of withstanding much harsher conditions such as high temperature, pressure, extreme pH and organic solvent, can be used as "artificial antibody", in place of natural biomolecules, applied in clinical analysis and diagnosis, chromatography separation, environmental monitoring and intelligent drug delivery systems[51,67−69].

For example, Miyata et al.[70] creatively used molecular imprinting in the presence of minute amount of cross-linkers to prepare molecular recognition responsive hydrogels and applied it in detection of α-fetoprotein (AFP, it is a glycoprotein widely used for serum diagnosis of primary hepatoma). In previously reported studies, molecular imprinting usually used large amounts of cross-linkers in order to obtain MIP with good mechanical property which is essential for MIP to retain the lefted template cavities with greater specific molecular recognition ability. However, the difficulty of molecular diffusion in such highly cross-linked MIP makes it difficult to remove the templets and may decrease the affinity capability to a great extent as well. Miyata et al. have surmounted these disadvantages by using minute amounts of cross-linkers and using interactions between template molecules (AFP) and the ligands as the additional cross-linking components. Thus, the gels could swell due to the decrease of crosslinking degree after removal of the template AFP, but recover to shrinkage by affinity binding AFP molecules. Therefore, the gels to undergo macroscopical volume changes induced by molecular affinity recognition may have potential applications in clinical molecular diagnostics.

4 Summary and outlook

Due to their smart behavior in response to environmental stimuli, intelligent hydrogels have significant potential applications in cell-cultured matrix, controlled drug delivery systems, tissue engineering and molecular diagnostics. Therefore, design and synthesis of new stimuli-responsive hydrogels will be applied in biomedical and biotechnological fields. The designed hydrogels are required to have suitable chemical properties, mechanical strength and biological functionalities. Hybrid hydrogels consisting of synthetic and naturally derived polymers or those composed of organic and inorganic components have received increasing attention, owing to their good mechanical properties and biological functions.

How to simply and effectively deliver the drugs to the predetermined pathological sites with less side effects and subsequently released drugs in an intelligent fashion are considered to be the key challenges for the applications of intelligent drug delivery system. Thus, synthesis of multi-functional responsive systems will be the first task in designing intelligent drug delivery systems. However, most of the reported work mainly focused on the physical-chemical sensitivity (such as pH, and temperature). Along with the combination of material science and biology, biosensitive drug carriers have attracted increasing attention. For example, polysaccharides and proteins can be used as biomarks for detection of physiological variations which may provide the possibility to release the drugs at the predetermined sites and tissue. It has been shown that intermolecular interactions, such as interaction between hemagglutinin and glucose and that between antigen and antibody, have provided advantageous tools to intelligent drug delivery systems. Thus, polymeric drug delivery systems based on the combination of biomolecules and stimuli responsive polymers may have wider applications in biomedical field. Additionally, the fundamental researches conducted on the drug delivery systems also facilitate us to better understand the biofunctions of biomacromolecules.

Polymers with molecular recognition ability can be achieved by introducing bioactive molecules to the polymer backbone, and then can be applied in different biomedical fields such as bioaffinity separation and molecular diagnostics. Although well defined polymer-protein conjugates have provided the possibilities to synthesize the excellent intelligent polymers with molecular recognition ability, the preparation methods and applications of such functional polymers are still limited at present. The synthesis of bioconjugated polymers with controlled and well defined structures is full of challenge. It is believed that with the development of the biotechnology, molecular biology and polymer chemistry, more and more polymers with molecular recognition ability will be synthesized and widely applied in biomedical fields. It is also worthy to note that the rapid development of glycomics in recent years has led to deeper understanding on the important role that carbohydrates molecules play a role in biological process including bacteria and virus infection, cell-cell interactions and signal transmission. However, it is difficult to synthesize and purify polysaccharides due to the multi-functionalities of the sugar unit (usually containing several hydroxyl groups) and the conformation variety of the oligosaccharides. Since glycopolymers may be prepared directly by polymerization methods and can mimic the function of polysaccharides to a certain degree, the development of novel glycopolymers is expected to be one of the crucial investigated fields in preparation of polymers bearing molecular recognition ability.

1 Peppas N A, Hilt J Z, Khademhosseini A, Langer R. Hydrogels in biology and medicine: From molecular principles to bionanotechnology. Adv Mater, 2006, 18(11): 1345－1360
2 Ganta S, Devalapally H, Shahiwala A, Amiji M. A review of stimuli-responsive nanocarriers for drug and gene delivery. J Control Release, 2008, 126(3): 187－204
3 Rijcken C J F, Soga O, Hennink W E, van Nostrum C F. Triggered destabilisation of polymeric micelles and vesicles by changing polymers polarity: An attractive tool for drug delivery. J Control Release, 2007, 120(3): 131－148
4 Hoffman A S. Bioconjugates of intelligent polymers and recognition proteins for use in diagnostics and affinity separations. Clin Chem, 2000, 46(9): 1478－1486
5 Gil E S, Hudson S M. Stimuli-responsive polymers and their bioconjugates. Prog Polym Sci, 2004, 29(12): 1173－1222
6 Yoshida R, Uchida K, Kaneko Y, Sakai K, Kikuchi A, Sakurai Y, Okano T. Comb-type grafted hydrogels with rapid deswelling response to temperature-changes. Nature, 1995, 374(6519): 240－242
7 Xu X D, Zhang X Z, Yang J, Cheng S X, Zhuo R X, Huang Y Q. Strategy to introduce a pendent micellar structure into poly(*N*-isopropylacrylamide) hydrogels. Langmuir, 2007, 23(8): 4231－4236
8 Gil E S, Hudson S M. Effect of silk fibroin interpenetrating networks on swelling/deswelling kinetics and rheological properties of poly(*N*-isopropylacrylamide) hydrogels. Biomacromolecules, 2007, 8(1): 258－264
9 Haraguchi K, Takehisa T, Ebato M. Control of cell cultivation and cell sheet detachment on the surface of polymer/clay nanocomposite hydrogels. Biomacromolecules, 2006, 7(11): 3267－3275
10 Zhang K W, Khan A. Phase-behavior of poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymers in water. Macromolecules, 1995, 28(11): 3807－3812
11 Zentner G M, Rathi R, Shih C, McRea J C, Seo M H, Oh H, Rhee B G, Mestecky J, Moldoveanu Z, Morgan M, Weitman S. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. J Control Release, 2001, 72(1-3): 203－215
12 Tate M C, Shear D A, Hoffman S W, Stein D G, LaPlaca M C. Biocompatibility of methylcellulose-based constructs designed for intracerebral gelation following experimental traumatic brain injury. Biomaterials, 2001, 22(10): 1113－1123
13 Scherlund M, Brodin A, Malmsten M. Nonionic cellulose ethers as potential drug delivery systems for periodontal anesthesia. J Colloid Interf Sci, 2000, 229(2): 365－374
14 Hoemann C D, Sun J, Légaré A, McKee M D, Ranger P, Buschmann M D. A thermosensitive polysaccharide gel for cell delivery in cartilage repair. Trans Orthop Res Soc, 2001, 26: 626－626
15 Kadlubowski S, Henke A, Ulanski P, Rosiak J M, Bromberg L, Hatton T A. Hydrogels of polyvinylpyrrolidone (PVP) and poly(acrylic acid) (PAA) synthesized by photoinduced crosslinking of homopolymers. Polymer, 2007, 48(17): 4974－4981
16 Khalid M N, Agnely F, Yagoubi N, Grossiord J L, Couarraze G. Water state characterization, swelling behavior, thermal and mechanical properties of chitosan based networks. Eur J Pharm Sci, 2002, 15(5): 425－432
17 Brahim S, Narinesingh D, Guiseppi-Elie A. Synthesis and hydration properties of pH-sensitive p(HEMA)-based hydrogels containing 3-(trimethoxysilyl)propyl methacrylate. Biomacromolecules, 2003, 4(3): 497－503
18 Park S E, Nho Y C, Lim Y M, Kim H I. Preparation of pH-sensitive poly(vinyl alcohol-*g*-methacrylic acid) and poly(vinyl alcohol-*g*-acrylic acid) hydrogels by gamma ray irradiation and their insulin release behavior. J Appl Polym Sci, 2004, 91(1): 636－643
19 Murthy N, Thng Y X, Schuck S, Xu M C, Frechet J M J. A novel strategy for encapsulation and release of proteins: Hydrogels and microgels with acid-labile acetal cross-linkers. J Am Chem Soc, 2002, 124(42): 12398－12399
20 Hamlen R P, Kent C E, Shafer S N. Electrolytically activated contractile polymer. Nature, 1965, 206: 1149－1150
21 Tanaka T, Nishio I, Sun S T, Ueno-Nishio S. Collapse of gels in an electric field. Science, 1982, 218: 467－469
22 Yao L, Krause S. Electromechanical responses of strong acid polymer gels in DC electric fields. Macromolecules, 2003, 36(6): 2055－2065
23 Kim S J, Kim M S, Kim S I, Spinks G M, Kim B C, Wallace G G. Self-oscillatory actuation at constant DC voltage with pH-sensitive chitosan/polyaniline hydrogel blend. Chem Mater, 2006, 18(24): 5805－5809
24 Desponds A, Freitag R. Synthesis and characterization of photoresponsive *N*-isopropylacrylamide cotelomers. Langmuir, 2003, 19(15): 6261－6270
25 Desponds A, Freitag R. Light-responsive bioconjugates as novel tools for specific capture of biologicals by photoaffinity precipitation. Biotechnol Bioeng, 2005, 91(5): 583－591
26 Zheng P J, Hu X, Zhao X Y, Li L, Tam K C, Gan L H. Photoregulated sol-gel transition of novel azobenzene-functionalized hydroxypropyl methylcellulose and its alpha-cyclodextrin complexes. Macromol Rapid Comm, 2004, 25(5): 678－682
27 Marchetti M. Thermodynamics of Temperature Sensitive Gels. Ph.D. Dissertation, University of Minnesota, 1989
28 Lee K K, Cussler E L. Pressure-dependent phase transitions in hydrogels. Chem Eng Sci, 1990, 45(3): 766－767
29 Shim W S, Yoo JS, Bae Y H, Lee D S. Novel injectable pH and temperature sensitive block copolymer hydrogel. Biomacromolecules, 2005, 6(6): 2930－2934
30 Bhattacharya S, Eckert F, Boyko V, Pich A. Temperature-, pH-, and magnetic-field-sensitive hybrid microgels. Small, 2007, 3(4): 650－657
31 Lee E S, Na K, Bae Y H. Polymeric micelle for tumor pH and folate-mediated targeting. J Control Release, 2003, 91(1-2): 103－113
32 Lee E S, Shin H J, Na K, Bae Y H. Poly(L-histidine)-PEG block copolymer micelles and pH-induced destabilization. J Control Release, 2003, 90(3): 363－374
33 Borchert U, Lipprandt U, Bilang M, Kimpfler A, Rank A, Peschka-Suss R, Schubert R, Lindner P, Forster S. pH-Induced release from P2VP-PEO block copolymer vesicles. Langmuir, 2006, 22(13): 5843－5847
34 Hu Y, Litwin T, Nagaraja A R, Kwong B, Katz J, Watson N, Irvine D J. Cytosolic delivery of membrane-impermeable molecules in dendritic cells using pH-responsive core-shell nanoparticles. Nano Lett, 2007, 7(10): 3056－3064
35 Sant V P, Smith D, Leroux J C. Enhancement of oral bioavailability of

poorly water-soluble drugs by poly(ethylene glycol)-block-poly(alkyl acrylate-*co*-methacrylic acid) self-assemblies. J Control Release, 2005, 104(2): 289－300
36 Sant V P, Smith D, Leroux J C. Novel pH-sensitive supramolecular assemblies for oral delivery of poorly water soluble drugs: Preparation and characterization. J Control Release, 2004, 97(2): 301－312
37 Sarmento B, Ribeiro A, Veiga F, Ferreira D, Neufeld R. Oral bioavailability of insulin contained in polysaccharide nanoparticles. Biomacromolecules, 2007, 8(10): 3054－3060
38 Lee Y, Fukushima S, Bae Y, Hiki S, Ishii T, Kataoka K. A protein nanocarrier from charge-conversion polymer in response to endosomal pH. J Am Chem Soc, 2007, 129(17): 5362－5363
39 Xu P S, van Kirk E A, Zhan Y H, Murdoch W J, Radosz M, Shen Y Q. Targeted charge-reversal nanoparticles for nuclear drug delivery. Angew Chem Int Ed, 2007, 46(26): 4999－5002
40 Liu S Q, Tong Y W, Yang Y Y. Incorporation and *in vitro* release of doxorubicin in thermally sensitive micelles made from poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide)-*b*-poly(D,L-lactide-*co*-glycolide) with varying compositions. Biomaterials, 2005, 26(24): 5064－5074
41 Sershen S R, Westcott S L, Halas N J, West J L. Temperature-sensitive polymer-nanoshell composites for photothermally modulated drug delivery. J Biomed Mater Res, 2000, 51(3): 293－298
42 Miyata T, Uragami T, Nakamae K. Biomolecule-sensitive hydrogels. Adv Drug Deliver Rev, 2002, 54(1): 79－98
43 Kim J J, Park K. Modulated insulin delivery from glucose-sensitive hydrogel dosage forms. J Control Release, 2001, 77(1-2): 39－47
44 Law C L, Gordon K A, Toki B E, Yamane A K, Hering M A, Cerveny C G, Petroziello J M, Ryan M C, Smith L, Simon R, Sauter G, Oflazoglu E, Doronina S O, Meyer D L, Francisco J A, Carter P, Senter P D, Copland J A, Wood C G, Wahl A F. Lymphocyte activation antigen CD70 expressed by renal cell carcinoma is a potential therapeutic target for anti-CD70 antibody-drug conjugates. Cancer Res, 2006, 66(4): 2328－2337
45 Iyer A K, Khaled G, Fang J, Maeda H. Exploiting the enhanced permeability and retention effect for tumor targeting. Drug Discov Today, 2006, 11(17-18): 812－818
46 Matsumura Y, Maeda H. A New Concept for Macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res, 1986, 46: 6387－6392
47 Yuan F, Dellian M, Fukumura D, Leunig M, Berk D A, Torchilin V P, Jain R K. Vascular-permeability in a human tumor xenograft-molecular-size dependence and cutoff size. Cancer Res, 1995, 55(17): 3752－3756
48 Peer D, Karp J M, Hong S, FaroKHzad O C, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. Nat Nanotechnol, 2007, 2(12): 751－760
49 Gu F X, Karnik R, Wang A Z, Alexis F, Levy-Nissenbaum E, Hong S, Langer R S, Farokhzad OC. Targeted nanoparticles for cancer therapy. Nano Today, 2007, 2(3): 14－21
50 Chandrasekar D, Sistla R, Ahmad F J, Khar R K, Diwan P V. The development of folate-PAMAM dendrimer conjugates for targeted delivery of anti-arthritic drugs and their pharmacokinetics and biodistribution in arthritic rats. Biomaterials, 2007, 28(3): 504－512
51 Bossi A, Bonini F, Turner A P F, Piletsky S A. Molecularly imprinted polymers for the recognition of proteins: The state of the art. Biosens Bioelectron, 2007, 22(6): 1131－1137
52 Goddard J M, Hotchkiss J H. Polymer surface modification for the attachment of bioactive compounds. Prog Polym Sci, 2007, 32(7): 698－725
53 Hoffman A S, Stayton P S. Bioconjugates of smart polymers and proteins: Synthesis and applications. Macromol Symp, 2004, 207: 139－151
54 Umeno D, Mori T, Maeda M. Single stranded DNA-poly (*N*-isopropylacrylamide) conjugate for affinity precipitation separation of oligonucleotides. Chem Commun, 1998, 14: 1433－1434
55 Klok H A. Biological-synthetic hybrid block copolymers: Combining the best from two worlds. J Polym Sci, Polym Chem, 2005, 43(1): 1－17
56 Stayton P S, Shimoboji T, Long C, Chilkoti A, Chen G H, Harris J M, Hoffman A S. Control of protein-ligand recognition using a stimuli-responsive polymer. Nature, 1995, 378(6556): 472－474
57 Boyer C, Bulmus V, Liu J Q, Davis T P, Stenzel M H, Barner-Kowollik C. Well-defined protein-polymer conjugates via *in situ* RAFT polymerization. J Am Chem Soc, 2007, 129(22): 7145－7154
58 Heredia K L, Bontempo D, Ly T, Byers J T, Halstenberg S, Maynard H D. *In situ* preparation of protein-"smart" polymer conjugates with retention of bioactivity. J Am Chem Soc, 2005, 127(48): 16955－16960
59 Heredia K L, Maynard H D. Synthesis of protein-polymer conjugates. Org Biomol Chem, 2007, 5(1): 45－53
60 Seeberger P H, Werz D B. Synthesis and medical applications of oligosaccharides. Nature, 2007, 446(7139): 1046－1051
61 Spain S G, Gibson M I, Cameron N R. Recent advances in the synthesis of well-defined glycopolymers. J Polym Sci, Polym Chem, 2007, 45(11): 2059－2072
62 Miura Y. Synthesis and biological application of glycopolymers. J Polym Sci, Polym Chem, 2007, 45(22): 5031－5036
63 Pasparakis G, Cockayne A, Alexander C. Control of bacterial aggregation by thermoresponsive glycopolymers. J Am Chem Soc, 2007, 129(36): 11014－11015
64 Dohi H, Nishida Y, Mizuno M, Shinkai M, Kobayashi T, Takeda T, Uzawa H, Kobayashi K. Synthesis of an artificial glycoconjugate polymer carrying P-k-antigenic trisaccharide and its potent neutralization activity against Shiga-like toxin. Bioorgan Med Chem, 1999, 7(9): 2053－2062
65 Owen R M, Gestwicki J E, Young T, Kiessling L L. Synthesis and applications of end-labeled neoglycopolymers. Org Lett, 2002, 4(14): 2293－2296
66 Polizzotti B D, Kiick K L. Effects of polymer structure on the inhibition of cholera toxin by linear polypeptide-based glycopolymers. Biomacromolecules, 2006, 7(2): 483－490
67 Alexander C, Andersson H S, Andersson L I, Ansell R J, Kirsch N, Nicholls I A, O'Mahony J, Whitcombe M J. Molecular imprinting science and technology: A survey of the literature for the years up to and including 2003. J Mol Recognit, 2006, 19(2): 106－180
68 Peppas N A, Huang Y. Polymers and gels as molecular recognition agents. Pharmaceut Res, 2002, 19(5): 578－587
69 Wei S, Mizaikoff B. Recent advances on noncovalent molecular imprints for affinity separations. J Sep Sci, 2007, 30(11): 1794－1805
70 Miyata T, Jige M, Nakaminami T, Uragami T. Tumor marker-responsive behavior of gels prepared by biomolecular imprinting. Proc Natl Acad Sci USA, 2006, 103(5): 1190－1193