Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation ☆

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ABSTRACT

Over the past 25 years many interesting biomedical uses have been proposed for stimuli-responsive polymers, including uses in diagnostics, drug delivery, tissue engineering (regenerative medicine), and cell culture. This article briefly overviews the field of stimuli-responsive polymers and describes some of the most successful biomedical applications to date of such "smart" polymers. Other interesting potential applications are also discussed. The major barriers to future clinical translation of smart polymers are also critically discussed.

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1. Introduction

Stimuli-responsive polymers are polymers that respond sharply to small changes in physical or chemical conditions with relatively large phase or property changes. Table 1 lists the various stimuli that can activate such dramatic behavior. These polymers are also variously referred to as "environmentally-sensitive", "smart" or "intelligent" polymers. Over the past 25 years they have been proposed for numerous biomedical uses, which are usually in an aqueous environment. When used as "smart biomaterials" they may be (a) dissolved in or phase-separated out of aqueous solutions, (b) adsorbed on or (c) chemically-grafted onto aqueous-solid interfaces, or the smart polymer molecules may be chemically cross-linked, H-bonded, and/or physically entangled in the form of (d) hydrogels. A number of reviews have highlighted potential applications of smart polymers in the biomedical field. The reader is referred to several chapters in the recently published 3rd edition of

Table 1
Environmental stimuli.

Physical
    Temperature
    Ionic strength
    Solvents
    Radiation (UV, visible)
    Electric field
    Mechanical stress
    High pressure
    Sonic radiation
    Magnetic field
Chemical
    pH
    Specific ions
    Chemical agents
Biochemical
    Enzyme substrates
    Affinity ligands

Fig. 1. Hypothetical structures and thermally-induced shrinking mechanisms for conventional PNIPAAm hydrogels compared to comb-grafted PNIPAAm gels. NG = Non-grafted Gel and GG = Grafted Gel, showing the molecular weight of the grafted PNIPAAm polymer. ([74], 1995, *Nature*, 374, 240).

the Textbook of Biomaterials Science ([59] and also to [13,14,75]; [15,45,61]).

Stimuli-responsive polymers may also be combined with a variety of bioactive molecules by physical mixing, chemical conjugation or complexation. These bioactive molecules include (a) proteins and peptides (e.g., enzymes, antibodies, growth factors, elastin-like peptides (ELPs) and the linker protein streptavidin); (b) nucleic acids (e.g., DNA, RNA and siRNA); (c) small organic molecules (e.g., steroids, cytotoxic drugs, anti-coagulants, anti-inflammatory drugs, biotin and cell membrane receptors); and (d) carbohydrates (e.g., heparin and hyaluronic acid). In addition, poly(ethylene glycol) (PEG) may be conjugated to or complexed with the smart polymer to provide it (plus any biomolecules combined with it) with "stealth" properties (e.g., [2,10,11,15,16]).

The most common smart polymer system that has been studied is the thermally-responsive smart polymer poly(N-isopropyl acrylamide), or PNIPAAm. (e.g., [12]). (See also Schild [76]). PNIPAAm sharply phase separates out of aqueous solution when the solution is warmed through a critical temperature (called the lower critical solution temperature, or LCST), which is ca. 32 °C in pure water, and a few degrees lower in physiologic saline. Above the LCST a highly wettable PNIPAAm-coated surface will suddenly become hydrophobic, and a crosslinked PNIPAAm hydrogel will sharply shrink and excrete its aqueous swelling solution. These phenomena all reverse when the stimulus is reversed, although reversal rates can vary widely depending on the geometry and composition of the smart polymer system (see below). Kinetics of the transitions also depend on structure in the case of hydrogels. Yoshida et al. [74] showed that macromonomers of NIPAAm could be used to form a water-swollen, crosslinked "comb-type" hydrogel. When that gel was heated above the LCST of PNIPAAm, it shrank much more rapidly than conventional crosslinked hydrogels of PNIPAAm, which collapsed slowly (Fig. 1).

The smart polymer may also respond to more than one stimulus, such as a copolymer of NIPAAm and acrylic acid (AAc), which is responsive to both temperature and pH. When NIPAAm-AAc copolymers were conjugated to a peptide site adjacent to the biotin binding site of streptavidin (SA), they showed a remarkable T/pH control of the binding of biotin to SA [77].

The temperature-stimulated phase separation of PNIPAAm was shown to be endothermic by [12]. Since it occurs spontaneously when temperature is raised through the LCST, the free energy drop must be driven by an entropy gain. Further, it is well-known that one can boil a solution of polyacrylamide and no phase separation will occur. Thus the temperature-induced phase separation of PNIPAAm must be due to an entropy gain related to the isopropyl groups of PNIPAAm. This is best understood as being driven by release of the hydrophobically-bound water molecules around the isopropyl
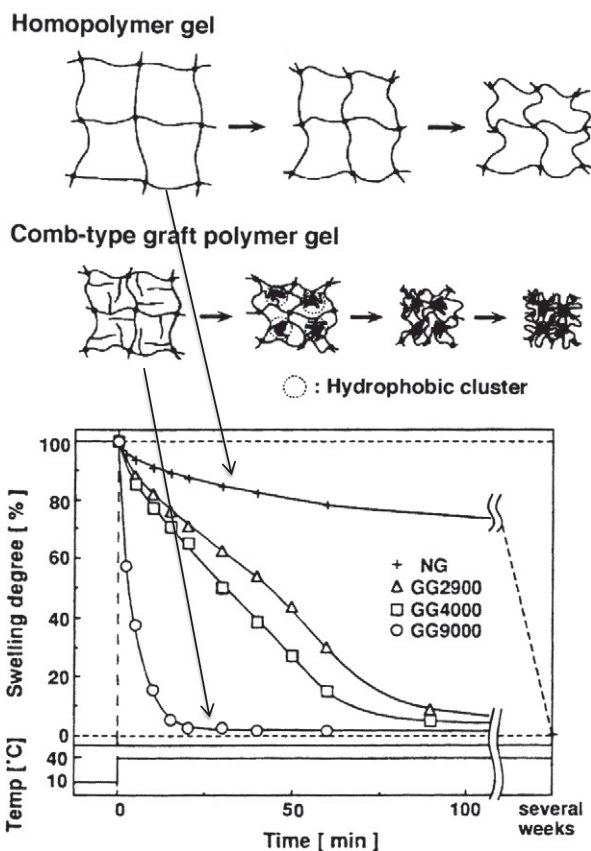
groups, as those groups aggregate together at the LCST. Often the rate of reversion back to the hydrated state may be slower than the collapse, because in the reverse process the hydrophobic groups of the polymer have to be rehydrated one by one, and that process is thermodynamically opposed by the resultant decline in entropy of the water molecules. The overall re-swelling process below the LCST is favored by the (small but positive) exothermic hydration of the hydrophobic groups and the gain in entropy as the polymer chain begins to expand. The rate of dehydration or rehydration of such smart polymer systems can also depend on the dimensions of the system. Phase-separation rates will be faster for smaller systems, i.e., nano-scale systems are faster than microscopic systems, which are, in turn, faster than macroscopic systems.

Thermally-responsive behavior may also be achieved by combining hydrophilic polymer components (such as PEG) with hydrophobic components (such as poly[lactic-co-glycolic acid] [PLGA]) (see work of S.W. Kim described below). The driving force behind the thermal response of such a smart triblock polymer is presumably similar to PNIPAAm, ie, it is due to the release of hydrophobically-bound water by the PLGA blocks, which then aggregate together.

2. Biomedical applications of stimuli-responsive polymers

There have been a number of interesting biomedical applications proposed for stimuli-responsive polymer systems, especially in the areas of drug delivery, cell culture surfaces, and diagnostics. Three of these have already been utilized in the clinic; they include the

following (Note: FDA-approved clinical trials and FDA-approved clinical use are both included here when using the term "clinical use"):

2.1. Enteric coatings on oral drug tablets

Enteric coatings on drug tablets have been available over-the-counter for more than 60 years. There are two main types of smart enteric polymer coatings used today. One is based on copolymers of pH-sensitive methacrylic monomers such as methacrylic acid (MAAc) and hydrophobic methacrylate monomers such as methyl methacrylate (MMA). Another type of enteric polymer is based on a cellulosic polymer backbone, where some of the $-CH_2OH$ groups are esterified with phthalic anhydride. Both types of polymers are hydrophobic at stomach or gastric pHs, since the carboxyl groups are protonated and non-ionized, and they become hydrophilic at intestinal or enteric pHs where the carboxyl groups are ionized. Thus, the drug is not released in the stomach, where it could irritate or inflame the stomach lining, but is rapidly released once it reaches the intestines where the pH rises to physiologic pH levels. The coatings are also useful for protecting 'fragile' drugs from stomach acid and gastric enzymes.

2.2. Smart cell culture surfaces

Okano and Yamato and co-workers have been pioneers in the area of "smart cell culture surfaces". [64,71]. Using an electron accelerator, they radiation-grafted PNIPAAm to polystyrene cell culture surfaces, and then cultured cells to confluent sheets on the surfaces at 37 °C, which is above the LCST of the polymer. At that temperature, the grafted PNIPAAm chains were collapsed, and the surface was hydrophobic, leading to physical adsorption of cell adhesion proteins from the culture medium, which enhanced the cell culture process as the cells grew to confluent cell sheets. Then, when the temperature was lowered below the LCST, the interface became hydrophilic as the PNIPAAm chains rehydrated, and the confluent cell sheets were released from the surface along with the cell adhesion proteins, which remained bound to the cell surfaces. They recently found that if the grafted NIPAAm chains were formed as brushes and terminated with COOH groups, the cells grew to confluence more rapidly at 37 °C, and they also released more rapidly and more cleanly from the surface at room temperature. [67]. These exciting new applications of smart surface cell sheets are being applied for corneal and myocardial tissue reconstruction by Okano and coworkers. (e.g., [64,71]).

Patterned PNIPAAm surfaces have also been prepared by [72] for other cell culture studies. Also, more recently the cell sheets have been deposited on each other layer-by-layer, to form multi-layered sheets with more than one type of cell. This yields a scaffold suitable for more complex tissue reconstruction of injured tissues.

2.3. Smart depot drug delivery systems (DDS)

SW Kim, et al. developed a family of thermally-gelling, hydrolytically-degradable tri-block copolymers that were physically mixed with drugs and injected sub-cutaneously or intra-muscularly to form phase-separated, degradable, drug depot masses at body conditions [17,23, 63,70]. These tri-block copolymers were composed of alternating ABA or BAB blocks such as PLGA-PEG-PLGA or PEG-PLGA-PEG blocks. The thermally-induced gelation of these block copolymer-drug mixtures is presumably driven by a similar mechanism to that of PNIPAAm, i.e., by the large entropy gain caused by the release of bound water molecules from the hydrophobic PLGA blocks at 37 °C. The PLGA blocks aggregate together and the phase-separated depot then slowly releases the drug by dissolution and diffusion of the drug, accompanied by and enhanced by the hydrolytic degradation of the PLGA-PEG-PLGA tri-block copolymer into PEG plus lactic acid and glycolic acid. These polymers have been tested in clinical trials by the company BTG in the UK; they carried out Phase 2 clinical trials for esophageal cancer treatment

with the drug paclitaxel, but at this time it is uncertain if they will proceed to Phase 3.

2.4. Smart mucosal drug delivery systems

Mucosal surfaces include the surface of the eye, the nasal membranes, portions of the gastro-intestinal tract, the anus, and the vagina, and together they represent potential targets for temperature- and/or pH-responsive polymer DDS. Mucoadhesive polymers are expected to enhance the residence time of the delivery formulation on the mucosal surfaces, where they may form physical hydrogels in response to the temperature and/or pH change upon contacting the surface. The gels may "bind" on contact with such surfaces via H-bonding interactions and physical entanglements between the mucoadhesive polymer and the mucin polymer molecules which coat mucosal surfaces. Mucins are known to contain a multiplicity of −COOH and −OH groups and much bound water. The mucins and the mucoadhesive polymers are both quite viscous, and they are both highly hydrated so that the physical interactions occurring during mucoadhesion are very complex. These physical interactions have been taken advantage of for delivering drugs from eye-drops into the eye or from nasal sprays into the nose with T-sensitive and mucoadhesive smart polymers, and from oral formulations in the stomach or intestines with pH-sensitive and muco-adhesive smart polymers (eg, Carbopol® [3]). Mucoadhesive polymers have also been proposed for use in vaginal drug delivery, where the pH is acidic in the vagina. Also some have proposed the use of pH-responsive polymers to deliver drugs within tumor tissues, which are slightly acidic. The most common mucoadhesive polymer is known as Carbopol®, which is a registered trademark of The Lubrizol Corporation for a family of polymers that are used as thickeners, suspending agents and stabilizers. They have been used in various vaginal drug delivery formulations in particular, to enhance residence time of the drug in the vagina. Most Carbopol® polymers are high molecular weight poly(acrylic acid) chains that are lightly crosslinked, and are available as powders or liquids (see [24] reference). Over the past 25 years many researchers have incorporated poly(acrylic acid) (PAAc) (or poly[methacrylic acid]) in their formulations to enhance drug delivery to the eye, nose, stomach or vagina [1,19,26,35,46–48,55–58,60,68].

3. Other smart polymers proposed for biomedical applications

There are many other interesting smart polymer systems that have been proposed for biomedical applications that have not yet been approved for clinical use. Some interesting examples are highlighted here.

3.1. Smart oral drug delivery system

An interesting pH- and temperature-responsive copolymer of NIPAAm and acrylic acid (AAc) was developed by [8] for use as an oral matrix drug delivery system (DDS). In this case, the copolymer was physically mixed with the drug, forming an *uncoated* matrix DDS. This matrix copolymer behaved similarly to the enteric copolymer coating, remaining insoluble at stomach temperature and acidic pHs, and later gradually dissolving in the intestines. However, an important difference was that the NIPAAm-AAc copolymer matrix released drug at intestinal pHs over several hours, and at rates that depended on the amount of AAc in the copolymer, as opposed to the enteric-coated tablet, where the drug would be rapidly released within the intestines once the coating dissolved. The mechanism behind the gradual swelling of the drug-loaded NIPAAm-AAc copolymer matrix at intestinal conditions (pH 7.3 and 37 °C) was a "competition" between the NIPAAm component of the copolymer that was resisting swelling above its LCST, while the AAc component of the copolymer was driving the swelling as the COOH groups became ionized at the increased pH of the intestines.

3.2. Smart, phase-separating depot drug delivery systems

Lee, Bae and coworkers [63] have developed degradable A-B-A triblock copolymers similar to those discussed in the previous section. These block copolymers have both pH- and temperature-sensitivity; they are based on blocks of poly(caprolactone-co-lactic acid) random copolymers (PCLA), sandwiching a central PEG block. The thermal sensitivity is similar to the PLGA/PEG block copolymers described above, while the pH-sensitivity is derived from the conjugation of short blocks containing the sulfonamide group (OSM) that are attached at each end of the triblock. Typical formulas of the copolymers are PCLA-PEG-PCLA and, when modified with oligomers containing the sulfonamide group at each end, OSM-PCLA-PEG-PCLA-OSM. [62,63].

3.3. Smart "elastin-like peptide" (ELP) biopolymers

These polymers are based on the temperature-induced phase separation behavior of a repeating peptide sequence within a hydrophobic domain of elastin. Elastin is a hydrophobic, crosslinked, elastic protein that has the unique mechanical property of repeated extensibility followed by 100% elastic recoil. The most common sequence in elastin is valine-proline-glycine-X-glycine, or (VPGXG)m, where X can be any amino acid other than proline, and m is the number of repeats. Elastin-like peptides (ELPs) have been studied extensively for biomaterial applications because they phase separate when heated, similar to PNIPAAm, but they are based on a natural protein and thus may be more "biocompatible" than a synthetic polymer, especially PNIPAAm. (See comments on this issue below). There are a number of other variants of ELPs that appear to exhibit elastin-like properties, but the pentapeptide motif of VPGXG has mostly been studied. Side groups capable of adding functionality to the ELP, e.g., cysteine, can be added to allow for conjugation of bioactive molecules, while lysine residues have been added to allow for crosslinking. Chilkoti and coworkers have extensively studied these smart polypeptides, e.g., [4,25,30,31]. These smart polypeptides may have some exciting applications as biomaterials in the future.

3.4. Smart, pH-responsive nanocarriers that enhance intracellular endosomal escape

Ethyl acrylic acid (EAAc) and propyl acrylic acid (PAAc) form pH-sensitive polymers and copolymers which become sharply hydrophobic as pH is lowered through their pKs, which is within the pH range of early endosomes. When these polymers are conjugated or complexed with drugs and endocytosed into target cells, they can disrupt the lipid bilayer of the endosome as pH drops within the endosome, enhancing "escape" of the polymer-drug carrier into the cytosol. [20,36–38,65,66,69,73]. This intracellular drug delivery technology has not yet been used in the clinic. Possibly if such smart materials and methods could show significantly enhanced delivery of drugs such as siRNA, they might be utilized in clinical testing.

Bae and coworkers have also developed interesting temperature- and pH-sensitive polymers useful for stimulating endosomal release of drug formulations. (e.g., [18,39]).

3.5. Smart diagnostic assays

One of the earliest applications of a smart polymer-biomolecule conjugate was an immunoassay developed by Hoffman, et al. in the mid-1980s. It was based on conjugation of an antibody to PNIPAAm. This smart bioconjugate was added to a blood test sample to capture an antigen such as a biomarker of hepatitis or AIDS, which were being screened in all blood banks at that time. After that, a second, labeled antibody was added, and that detection antibody was designed to affinity-link to the same antigen. Finally, the solution was warmed to phase-separate the labeled immune complex sandwich. The assay resembled an ELISA assay, except that it was run in solution, with a last, phase-separation step. In contrast to a typical ELISA multi-well plate assay, it was much faster and just as accurate [33,34,78,79]. However, even though close to $25 million was raised in 1986 for a new company that was dedicated to apply the smart diagnostic test for screening blood in blood banks, it did not become commercialized due to time and cost issues.

Smart, PNIPAAm diagnostics technology has more recently been applied by Stayton & Hoffman, et al. to several novel surface and nanoparticle-based diagnostic systems that use PNIPAAm coatings on microfluidic channels, on gold nanoparticles and on magnetic nanoparticles. [9,21,22,27–29,42–44]. These smart nano-scale systems are being designed and developed for clinical immunoassays. Figs. 2 and 3 describe one such immunoassay.

3.6. Smart hydrogels for drug delivery

When a smart polymer is cross-linked to form a gel, the gel will collapse and re-swell in water as a stimulus raises or lowers it through its critical condition. If a drug is loaded into the gel, the collapse can release
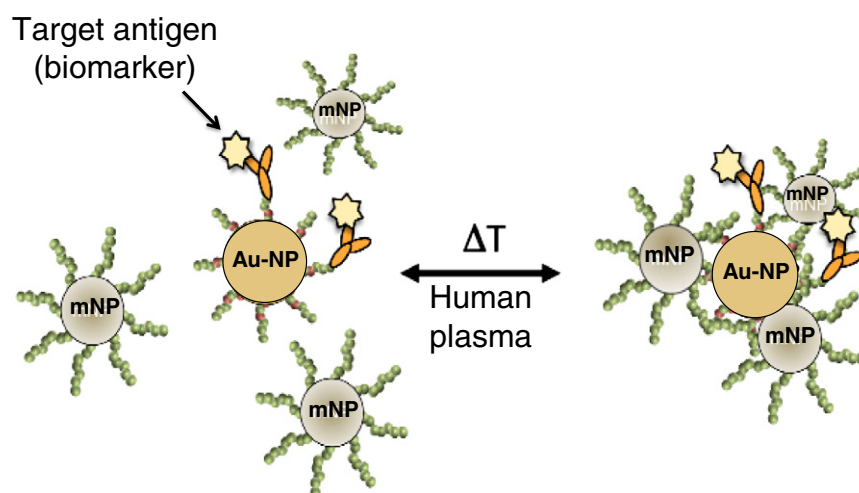

Fig. 2. When PNIPAAm is coated on magnetic nanoparticles (mNP) and gold nanoparticles (Au-NP), it acts to aggregate or "glue" the NPs together when the temperature is raised above the LCST of PNIPAAm. ([43], *Nano Letters*, 10, 85–89).
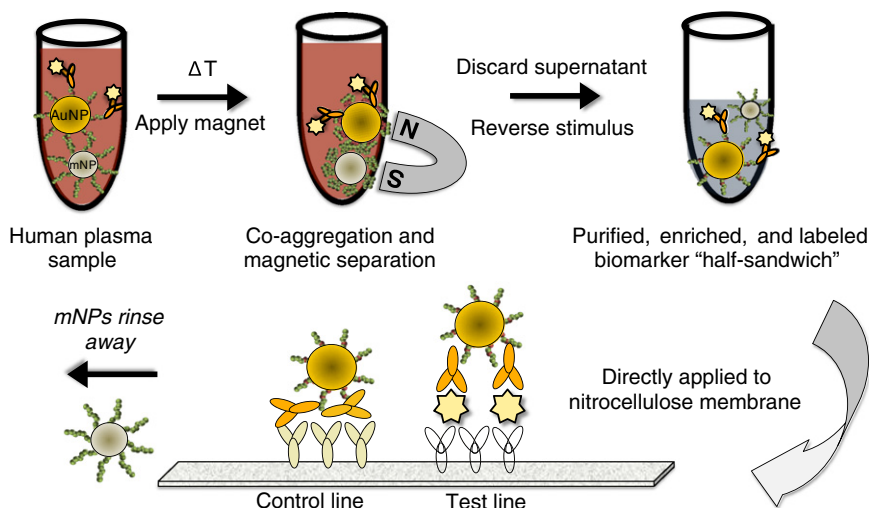
Fig. 3. Biomarkers in blood plasma test samples at RT are captured by antibodies bound to PNIPAAm-coated gold nanoparticles (NPs), which are then thermally-aggregated with PNIPAAm-coated magnetic NPs and isolated and concentrated by a magnetic field. After washing the aggregates and then lowering the temperature below the LCST, the NPs are dispersed and flowed onto a lateral flow strip for biomarker assay. ([43], *Nano Letters*, 10, 85–89).

the drug in a burst. Hoffman and co-workers were among the first to recognize the potential of PNIPAAm hydrogels as biomaterials; they showed that smart gels could be used to entrap drugs and deliver them. They also entrapped enzymes and cells in smart gels, and by inducing cyclic collapse and swelling of the gel, the enzymes (or enzymes within the cells) could be turned "on" and "off". [5–8,49–54].

Kim, Okano, Bae and co-workers also actively studied smart hydrogels in the late 1980s and 1990s. For example, they investigated smart gels containing entrapped cells that could be used as "artificial organs" [70]. Peppas and co-workers have extensively studied pH-sensitive acrylic acid-acrylate copolymer smart gels for drug delivery. [55–58,60]. Nakamae, Hoffman, and co-workers developed novel compositions of smart gels containing phosphate groups that were used to bind cationic proteins as model drugs, which were then released by a combination of thermal stimuli and ion exchange [32,40,41].

4. Future challenges for clinical translation of stimuli-responsive polymers

There are many current "smart" polymer drug delivery, diagnostic and tissue engineering applications that have not yet made it into the clinic. Some of the reasons for this are best understood in terms of the potential cellular toxicity of the smart polymers, especially for applications involving intracellular delivery of biomolecular drugs such as peptide, protein and nucleic acid drugs, which mainly act within cells. Many of the smart carrier systems utilize acrylamide or acrylic acid type polymers such as PNIPAAm and PPAAc, which are not hydrolytically degradable. Concerns about the potential toxicity of PNIPAAm have often been assumed but to the author's knowledge, not yet clearly elucidated. The concerns may derive from the known neurotoxicity of AAm monomer. There is little likelihood that NIPAAm monomer is present in PNIPAAm formulations that have been studied because they are usually thoroughly washed before incorporating drugs. Thus, the possibility that PNIPAAm itself is toxic still remains to be properly studied in cell culture and in animal testing, i.e., "preclinical" implant studies. Frankly, this author believes that PNIPAAm has been unfortunately "dismissed" as having a high potential for toxicity, based on the neurotoxicity of AAm monomer.

Further, many of the smart polymer carriers are most effective in reaching their cellular targets when higher molecular weights are used; such polymers are not readily excreted via the kidneys after

delivering the drug, and the polymers are not biodegradable, so they would tend to accumulate in the body. That could be another important reason they have not been tested in clinical trials. Clinical trials are very expensive, and companies are reluctant to invest in such expensive testing if there is a real potential for toxicity, and prefer to depend on known, "FDA-friendly" polymers such as PEG and PLGA. If the drug is an antigen and the purpose is to use it as a "one or two shot vaccine", use of a smart polymer carrier might succeed in the clinic if the vaccination efficacy is much better than current practice, but that may not be a great enough incentive to go through clinical trials because the dose of a simple injection of the antigen may always be increased. However, if the cost of the antigen becomes prohibitive, and the smart polymer carrier formulation is much more effective than any others, then there might be a better chance for the smart polymer vaccine formulation to be approved for clinical vaccinations.

Other reasons that smart polymer DDS have rarely been used in the clinic may be that simple injection of the biomolecular drug without a carrier is just that, a simple and well-accepted drug delivery method. While in the special case of cancer (where a large fraction of the current smart DDS research is focused) smart polymer delivery systems may offer attractive features such as special protection of the drug during circulation, plus physical targeting (EPR) and/or active targeting with ligands to reach the target cancer cells, but they may not be successful in entering the clinic unless they offer a unique possibility that every last cancer cell can be killed. Some cancer cells can survive as dormant and inaccessible cells, while others metastasize and are very difficult to kill. These challenging behaviors of cancer cells towards any kind of therapy represent another huge barrier to clinical use of polymer carriers in general, as well as new smart polymer carriers.

Oral delivery of biomolecular drugs is an attractive delivery route, but it is inefficient due to the difficulty of protecting those drugs from gastric acid and enteric enzymes. The Dong & Hoffman oral delivery system described above, based on a smart pH- & temperature-sensitive matrix for oral delivery of acid- and enzyme-sensitive drugs may find a clinical application for oral delivery if the polymer can be shown to be non-toxic as it passes through the GI tract.

Smart diagnostic applications, rather than drug delivery applications, are much more likely to succeed in the clinic, since their use does not normally involve direct contact with the body. This is an area that holds huge potential for smart polymer applications, and it should eventually be very successful.

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