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Review Article

HYDROGELS AS A DRUG DELIVERY SYSTEM AND APPLICATIONS: A REVIEW

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ABSTRACT

Once used for simple encapsulation of cells or drugs, in homogenous materials, today's hydrogels are more complex smart polymers with different types of ligands and cross links allowing for highly regulated structures and different bioresponsive functionalities. This review will emphasize on the various aspects of the hydrogels. Hydrogels that undergo swelling changes in response to specific biomolecules have become increasingly important because of their potential applications in the development of biomaterials and drug delivery systems and the biomedical applications of the hydrogels. We concentrate on the bioresponsive hydrogels and sensors, also introduces the physical background of the special properties of stimuli-responsive hydrogels; use of the various transducers describing changes in physical properties of the hydrogels. This paper considers the possibility of using artificial neural network models to identify model for swelling behavior as new techniques.

Keywords: Hydrogel, Biomolecules, Stimuli-responsive.

INTRODUCTION

Hydrogels are crosslinked hydrophilic polymers capable of imbibing large volume of water but insoluble in water because of their network structure. Hydrogels are hydrophilic polymer networks which may absorb from 10–20% up to thousands of times their dry weight in water. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve. They are called 'reversible' or 'physical' gels when the networks are held together by molecular entanglements [1]. Till date two types of hydrogels are present in market which includes physical and chemical hydrogels. Physical hydrogels are not homogeneous because, in homogeneity cluster of molecular entanglement and ionically associated domains created. Free chain ends or chain loops represent transient network defects in physical gels. Physical hydrogel formed when a polyelectrolyte is combined with a multivalent ion of the opposite charge, known as an 'ionotropic' hydrogels e.g calcium alginate hydrogels. In some cases polyelectrolyte of different Charges are mixed and gel was formed by poly-ions or complex coacervates [2].These interactions are affected by change in the ionic strength, pH, temperature, applications of stress or addition of the solute that competes with polymeric ligands for the affinity site on the protein. Chemical hydrogels may generate by cross linking of water-soluble polymers or by conversion of hydrophobic polymers to hydrophilic polymers plus cross linking to form a network. Hydrogels when covalently cross linked networks called 'permanent' or 'chemical' gels. Chemical hydrogels may also be generated from cross-linking of water-soluble polymers, or by conversion of hydrophobic polymers to hydrophilic polymers with cross- linking to form a network. Chemical hydrogels are also not homogeneous. They usually contain regions of low water swelling and high crosslink density, called 'clusters', which are dispersed within regions of high swelling, and low crosslink density[3].

Applications of Hydrogels

Hydrogels for Inflammatory conditions

During inflammation, hydroxyl radicals (OH) are produced from inflammation cells. Yui and co-workers [4] focused on the inflammatory-induced hydroxyl radicals and developed drug delivery systems, which related to the hydroxyl radicals. They used hyaluronic acid (a linear mucopolysaccharide composed of repeating disaccharide subunits of *N*-acetyl-D-glucosamine and D-guluronic acid). In the body, HA is mainly degraded either by a specific enzyme, hyaluronidase, or hydroxyl radicals. Degradation of HA via the hyaluronidase is very low in a normal state of health. Degradation via hydroxyl radicals however, is usually dominant and rapid when HA is injected at inflammatory sites. Thus, Yui and co-workers prepared cross-linked HA with ethylene glycol diglycidylether or polyglycerol polyglycidylether [5]. These HA gels degraded only when the hydroxyl radicals were generated by the Fenton reaction between Fe ions and hydrogen peroxide *in vitro*. Thus, a surface erosion type of degradation occurs. When microspheres were incorporated in the HA hydrogels as a model drug, these microspheres were released only when hydroxyl radicals induced HA gel degradation. The microsphere release was regulated by the surface erosion type of degradation.

Thermoresponsive hydrogel systems

Akihiko and Truo Okano [6] in the pulsatile drug release control using hydrogel explain thermoreresponsive hydrogel system. Thermoresponsive hydrogels as the carrier for the Stimuli-responsive drug delivery system. PIPAAm cross-linked gels have thermoresponsive, discontinuous swelling/deswelling.

A sudden temperature rise above the transition temperature, these gels resulted in the formation of a dense shrunken layer on the gel surface ('skin layer'), which hindered water permeation from inside the gel into the environment. Drug release from the PIPAAm hydrogels at temperatures below 32^0C was governed by diffusion, while above this temperature drug release was stopped completely, due to the 'skin layer' formation on the gel surface (on–off drug release regulation).

Bioresponsive hydrogels for drug delivery system

Much work on bioresponsive hydrogels for drug delivery relates to the release of insulin in response to raised blood sugar levels [7]. In one approach, glucose oxidase molecules are immobilized onto a basic polymeric carrier. Following the enzyme reaction that converts glucose to gluconic acid, thereby temporarily lowering the pH, the basic groups on the polymer are protonated, inducing swelling and enhancing the release profile of insulin. This system works as a feedback loop, upon release of insulin the sugar levels drop, resulting in a pH increase that stops the release of further insulin.

Ishihara *et al.* [8] combined a copolymer membrane of N, N-diethylaminoethyl methacrylate (DEA) and 2-hydroxypropyl methacrylate (HPMA) with a cross-linked poly (acrylamide) membrane, in which glucose oxidase was immobilized. The glucose-sensitive insulin permeation was achieved based upon the combination of an enzymatic reaction with a pH-sensitive swelling. In this system, glucose diffuses into the membrane and is catalyzed by glucose oxidase, resulting in the conversion of glucose to gluconic acid. The micro environmental pH in the membrane becomes low, due to the production of gluconic acid. As the membrane swells, resulting from ionization of the amine groups by the lower pH, insulin permeability through the membrane is enhanced. Thus, insulin permeation through the membrane is strongly dependent

upon the glucose concentration. Further, Ishihara *et al.*[8] investigated insulin release from polymer capsules containing insulin and glucose oxidase, which were prepared by a conventional interfacial precipitation method. Insulin release was inhibited in the absence of glucose, but was strongly enhanced in the presence of glucose. In case of site-specific release explains the catalytic action of disease-specific enzymes to trigger drug release from polymeric prodrug carriers. Prodrugs are inactive precursors of drug molecules that are activated *in vivo*, usually through enzymatic hydrolysis. For example, a cancer-specific enzyme secreted by tumor cells can be used to trigger the release of a therapeutic agent to prevent or reduce metastasis (targeted chemotherapy). This objective may be achieved by immobilizing drug molecules linked to a polymeric backbone (such as polyethylene glycol, or PEG) via enzyme-cleavable linkers. Rein V.ULijnl *et al*. [7] developed a nondissolving, enzyme-responsive hydrogel with physically entrapped guest molecules. Macromolecule release is determined by charge-induced hydrogel swelling, which is controlled enzymatically. A cleavable peptide chain is modified to respond to a particular protease.

Glucose sensitive Hydrogels

Hydrogels with lectin**-** Lectins are carbohydrate-binding proteins, interact with glycoprotein's and glycolipids on the cell surface and cause various effects, such as cell agglutination, cell adhesion to surfaces, and hormone-like action. The unique carbohydrate-binding properties of lectins are very useful for the fabrication of glucose-sensitive systems. Therefore, some researchers have focused on the glucose-binding properties of concanavalin A (Con A), a lectin possessing four binding sites. Brownlee et al.[9] and Kim et al.[10] involved in development of glucose-sensitive insulin release systems using Con A. Their strategy was to synthesize a stable, biologically active glycosylated insulin derivative able to form a complex with Con A. The glycosylated insulin derivative could be released from its complex with Con A in the presence of free glucose, based on the competitive and complementary binding properties of glycosylated insulin and glucose to Con A.

Hydrogels with phenylboronic acid moieties

Phenylboronic acid and its derivatives [11] form complexes with polyol compounds, such as glucose in aqueous solution. The complex between phenylboronic acid and a polyol compound can be dissociated in the presence of a competing polyol compound which is able to form a stronger complex. This means that complex formation between phenylboronic acid and a polyol compound has many potential applications as a glucose-sensitive material. Kitano *et al*. [12] synthesized copolymers with phenylboronic acid moieties (poly (NVP-*co*-PBA)) by copolymerizing *N*-vinyl-2-pyrrolidone (NVP) and 3-(acrylamido) phenylboronic acid (PBA). Due to the reversible complex formation between phenylboronic acid of poly (NVP-*co*-PBA) and poly (vinyl alcohol) (PVA), the competitive binding of phenylboronic acid with glucose and PVA could be utilized to construct a glucose-sensitive system. The formation and dissociation of the poly (NVP-*co*-PBA) /PVA complex could be investigated by observing the change in viscosity. Viscosity measurements revealed that poly (NVP-co-PBA) formed a complex with PVA in the absence of glucose; however the complex dissociated in the presence of glucose.

Bioresponsive hydrogels for sensing

In these systems, a biological recognition event is coupled to a macroscopically observable change in hydrogel properties. Holtz and Asher [13] have developed a hydrogel-based photonic crystal that acts as a glucose sensor for patients with diabetes mellitus. Glucose oxidase is attached to arrays of polystyrene nanospheres, which are then polymerized within a hydrogel matrix. The resulting material reversibly swells in the presence of glucose, similar to the glucose-responsive systems described earlier. The swelling increases the mean separation between the immobilized nanospheres, shifting the Bragg peak of diffracted light to longer wavelengths and producing a red-shift in the optical properties (i.e. a readily observed color change) of the polymer. This system can be implanted as contact lenses or ocular inserts to detect small changes in blood glucose levels indirectly via tear fluid. In this modified system, boronic acid derivatives are attached to the array and polymerized within a network of polyacrylamide PEG. Glucose binds to the derivatives, producing cross-links that shrink the hydrogel and cause a blue-shift. The patient is then able to determine their blood glucose levels via a color chart.

Recent development in the hydrogel based biosensor [7]

Stimulus	Hydrogel	Application	Output signal
Glucose	PA-PEG	Glucose biosensor	Optical, color
Protein	PNIPAmcoAAc	Avidin, Antibiotin Biosensor	Optical, Focusing
Peptide	PEG	Live cell biosensor	Biochemical, Fluorescence

Protein Sensitive Hydrogels

Hydrogels sensitive to enzymes

The microbial enzymes that are predominantly present in the colon can be used as signals for site-specific delivery of drugs to the colon. Hovgaard *et al*. [14] focused on the fact that microbial enzymes in the colon, such as dextranases, can degrade the polysaccharide dextran. They prepared dextran hydrogels cross-linked with diisocyanate for colony-specific drug delivery. The dextran hydrogels were degraded *in vitro* by a model dextranase, as well as *in vivo* in rats and in a human colonic fermentation model. Release of a drug from the dextran hydrogels can be controlled by the presence of dextranase. Above discussion shows that enzymes play important role in the design of the hydrogels.

Azoreductase is also useful for colon-specific drug delivery as it is an enzyme produced by the microbial flora of the colon, to construct colon-specific drug delivery systems, a few researchers used azo aromatic bonds, which can be degraded by azoreductase [15].

Bioresponsive hydrogels (used in tissue engineering)

Tissue engineering had potential to regenerate damaged or diseased tissues and organs. The development of biomaterials that facilitate the mechanical and cellular regeneration of tissue is crucial to its success. Current strategies involve the production of porous scaffolds for cells to colonize. Ideal scaffolds are those that mimic the ECM that surrounds cells in their natural context. The current trend is on creating materials that are highly hydrated, nanofibrous, directional, of appropriate mechanical strength, and contain bioactive signals to direct cell behavior. Here, we cover recent research on materials that respond to (cell-secreted) enzymes or are modified by enzymatic action, thereby mimicking the adaptive properties of natural ECMs.

ECM-mimicking hydrogel scaffolds that permit cell migration have been studied by Hubbell and coworkers [16]. The researchers use oligopeptides as cross-linkers in PEG-based hydrogels. The peptide sequences are cleavable by matrix metalloproteinase's (MMPs) to form a gel into which cells can infiltrate. MMPs are a family of enzymes causes the breakdown of ECM molecules during tissue remodeling and disease. Therefore, the integration of MMP-cleavable sites is a logical approach toward ECM mimics. Human fibroblasts are encouraged to invade the hydrogel through integrinbinding domains (Arg-Gly-Asp-Ser-Pro) that are incorporated via PEG linkers. The fibroblasts then cause a local breakdown of the hydrogel cross-links via secreted MMPs. In bone tissue engineering, tested by loading the gel with bone morphogenetic protein-2 (BMP-2), which is known to be involved in bone formation. An assessment of the degradation behavior of MMPs and the cell invasion of provisional matrices revealed that the healing response *in vivo* depends on the enzymatic sensitivity of the matrix.

There are some formulations of injectable hydrogel of PNIPAm-co-AAc to mimic the ECM. These hydrogels are prepared by cross-linking an MMP-13/collagenase-3-degradable peptide sequence and NIPAm in the presence of Arg-Gly-Asp-modified poly (AAc). The proteolytic degradation and cell adhesion properties of this hydrogel were studied using rat calvarial osteoblasts. Collagenase was found

to degrade the hydrogel, with the rate dependent on the concentration of collagenase in relation to the poly (AAc) chain. However, greater migration is seen in those hydrogels that contain Arg-Gly-Asp. There is also an increase in cell migration in MMP-degradable hydrogels compared with nodegradable gels, indicating the advantage of bioresponsive hydrogels.[16]

Hydrogels sensitive to Antigen

The specific antigen-recognition function of an antibody can provide the basis for constructing sensors with various uses for immunoassays and antigen sensing. This section describes novel antigen-sensitive hydrogels that undergo swelling changes in response to a specific antigen [11]. Antigen-sensitive hydrogels were prepared by using antigen–antibody bonds at cross-linking points in the hydrogels [17]. E.g. rabbit immunoglobulin G (IgG), the antigen, was chemically modified by coupling with *N*-succinimidylacrylate (NSA) in phosphate buffer solution to introduce vinyl groups into the rabbit IgG. The resultant vinyl rabbit IgG was mixed with the antibody, goat anti rabbit IgG (GAR IgG), to form an antigen–antibody complex. The vinyl-rabbit IgG was then copolymerized with acrylamide (AAm) as a co-monomer and N, N 9-methylenebisacrylamide (MBAA) as a cross-linker in the presence of GAR IgG, resulting in a hydrogel containing antigen–antibody bond sites (antigen–antibody entrapment hydrogel). The antigen-sensitive swelling of the antigen–antibody entrapment hydrogel [11] can be explained by the complex exchange mechanism as follows: in the antigen-antibody entrapment hydrogel in a buffer solution containing a free antigen, the free antigen induces the dissociation of the antigen–antibody bonds grafted to the network, due to the stronger affinity of the antibody for the free antigen than for the antigen grafted to the network. Therefore, the hydrogel underwent swelling in the presence of the free antigen because the dissociation of the antigen antibody bonds resulted in a decrease in the cross linking density. Thus, the antigen–antibody entrapment hydrogel showed antigen-sensitive behavior on the basis of the competitive binding properties of the free antigen and network-grafted antigen to antibody.

Hydrogels used as carrier in controlled drug delivery system

Hydrogels can be classified as neutral, anionic or cationic. The drug release behaviour and associated swelling characteristics of hydrogels are the result of cross links (otherwise known as tie-points or junctions), permanent entanglements, ionic interactions, or microcrystalline regions incorporating various chains [18]. Hydrogels have been used as carriers for pharmaceutical applications, such as carriers for delivery of drugs, peptides or proteins .They have been used to regulate drug release in reservoir-based, controlled release systems or as carriers in swellable and swelling-controlled release devices.

The development 'conventional' controlled release devices based on hydrogels or hydrophilic carriers that can swell in the presence of a biological fluid [19]. Solvent-activated systems include osmotic-controlled and swelling-controlled release systems. The overall rate of drug release is controlled by the rate of water influx. In swelling-controlled systems, the drug, which is dispersed in the polymer, diffuses out as water uptake occurs and the polymer swells. The drug release rate is dependent both on water diffusion and polymer chain relaxation. Continued swelling of this system results in the drug diffusing out at a faster rate with the rate of carrier swelling controlling the overall drug release rate. The time dependence of the rate of drug release can be determined depending on the rate of water diffusion and chain relaxation [14].

In addition, new technology of hydrogels was developed by Park and collaborators [20]. They mentioned the preparation of superporous hydrogels and composites from a wide polymers including poly (acrylic acid) (PAA), PIPAAm. The highly porous and well-structured porous network can be used for the relatively fast release of a wide range of drugs or proteins. Such developments can lead to improved drug delivery systems.

There have been numerous new or improved pharmaceutical applications of hydrogels. Of particular interest, have been the applications of poly (ethylene glycol) (PEG) in such applications [21]. One of the most interesting properties is that PEG resists recognition from the immune system. It avoids protein and cell adsorption.

Molecular imprinting within hydrogels

Molecular imprinting involves forming a pre-polymerization complex between the template molecule and functional monomers or functional oligomers (or polymers) [2] with specific chemical structures interact with the template either by covalent [22], non-covalent chemistry [23]. Once the pre-polymerization complex is formed, the polymerisation reaction occurs in the presence of a cross linking monomer and an appropriate solvent which controls the overall polymer morphology and macro porous structure. Once the template is removed, the product is a hetero polymer matrix with specific recognition elements for the template molecule. Several reviews exist explaining the upcoming field of molecular imprinting and designed molecular recognition[24].The macromolecular architecture must be designed differently than more traditional dense network and must include a spatially varying cross-linking density (micro and macroporous regions). A density fluctuation in the polymer network generates regions or microgels of localized higher cross linking, which contain an effective imprinting structure and proper rigidity to produce adequate specificity (areas or patches of recognition).

Designing the network for hydrogels has also shifted focus towards more traditional rigid body approaches to imprinting with regards to the cross linking and functional monomer ratio. Hence, there is an optimum cross linking to functional monomer molecular mass ratio, which directly depends on the size of the template. For the types of small molecular mass molecules imprinted to date, ethylene glycol, dimethacrylate has been the most favoured and successful choice with currently available functional monomers [25]. It is easy to speculate that imprinted gels or chains possessing certain macromolecular architecture with binding abilities could be used as the sensing elements within Analyte sensitive controlled release systems. Analyte sensitive polymer networks have been the focus of much research (mostly saccharide recognition) and have been designed in a number of ways. They have included enzymes, which as a result of reaction invoke a local pH change, modulating the swelling of the network and thus release. Intelligent analytical-sensitive hydrogels network present showing action by induced swelling, Loss of effective cross-links [25]. Some author has recently described the immunogenicity of the phenylboronate moiety, which may recognize *N*-acetylneuraminic acid residues on the plasma membrane of lymphocytes. Imprinting structures could be used in these circumstances to greatly decrease cross reactivity and non-specific binding.

Increasing application of Hydrogels in biological sensing due to

1. Biological molecules can be covalently incorporated into hydrogel structures.
2. Hydrogels mechanical properties are highly tunable also elasticity can be tailored by modifying cross-link densities.
3. Hydrogels provide suitable semi wet and three-dimensional environments for molecular-level biological interactions.
4. Many hydrogels shows inert surfaces that prevent nonspecific adsorption of proteins, known as antifouling.
5. Hydrogels can be designed to change properties due to externally applied factors, such as temperature, ionic strength, solvent polarity, electric /magnetic field, light, or small biomolecules.

There is availability bioresponsive hydrogels. Three types of stimuli for bioresponsive hydrogel [7] systems can be distinguished. At start, hydrogel materials modified to contain small biomolecules that selectively bind to biomacromolecules, including protein receptors or antibodies. Upon binding, a macroscopic transition takes place. Also, systems may be modified with enzyme-sensitive substrates, such as short peptides. Here, the initial molecular recognition event is similar to that in the enzyme protein binds to substrate, followed

by a chemical event involving the making or breaking of bonds within the enzyme-sensitive substrate. Third, systems may have biomacromolecules, such as enzymes, incorporated into their structures that recognize small biomolecules.

Now days, hydrogel research is in situ hydrogel formation by photo polymerization or by phase transition [20, 26]. A particularly interesting and important polymeric system is hydrogel forming solutions by a simple phase transition (sol–gel transition) in water without any chemical reaction or external stimulation. This system provides simplicity and safety in *in vivo* situations. The sol-to-gel transition of aqueous polymer solutions induced by temperature will be stressed, covering the natural or semi natural polymeric systems [27].

In hydrogels cross linking is generally present in order to prevent dissolution of the hydrophilic polymer chains in an aqueous media. Hydrogels possess a good biocompatibility; their hydrophilic surface has a low interfacial free energy in contact with body fluids which result in low tendency for proteins and cells to adhere to these surfaces [28]. Also, soft and rubbery nature of the hydrogels reduces irritations to the adjacent tissues [29]. There are stimuli-sensitive hydrogels are present which can change their volume in the response to the small change in the certain environmental parameters [30].So, much of the focus is given to the development of the stimuli-sensitive hydrogels with sensitivities across temperature [31], electric field value [32], light [33], PH. Solvent composition and specific ions [34].This is useful for the use of the hydrogels in the pharmaceutical manufacturing of the dosage forms.

Hydrogels have application in the nanoparticles. Biodegradable polyester nanoparticles are carrier for low molecular mass drugs [35]. The nanoparticles system has wide range of applications. First, nanoparticles, have small size, penetrate within even small capillaries and are taken up within cells, which help for efficient drug accumulation at the target sites in the body[36]. Second, the use of biodegradable materials for nanoparticles preparation allows for sustained drug release within the target site over a period of days or even weeks after injection [37]. Also, there is availability of the imprinting gel system are intelligent, stimuli-sensitive gels that modify their swelling behavior and in turn modulate Analyte binding abilities [25]. This review is focusing on the wide range of the applications of the hydrogels and nature of the hydrogels.

Dispersed hydrogel networks (Nanogel and Microgel)

A gel is a three-dimensional cross-linked polymer network immersed in a fluid. Dispersed gel particles. Microgels and nanogels can be viewed as cross-linked latex particles, which are swollen by a good solvent. If the good solvent is water these species belong to a hydrogel class [38]. Colloidal stability of such systems results primarily from matching of the Hamaker constants of the swollen particles and the solvent. These systems are currently being actively investigated due to their potential technological applications in a large number of areas: medicine, industry, and environmental cleanup. Applications of such materials include surface coating, uptake and release of heavy metal ions, drug delivery, optoelectronic switches, and others. The research in these fields is propelled by the interest in the ability of hydrogel materials to respond reversibly to external stimuli, such as temperature, pH, ionic strength, solvent nature, and external stress. The microgel and nanogel particles are of particular interest because they exhibit intrinsic properties of gels combined with the properties of colloids, such as micro heterogeneous structure, small size and high surface to volume ratio.

Various ideas can be used for preparation of both bulk (macrogels) and dispersed gels. Macrogels are prepared using solutions in the presence of cross-linker and an accelerator. The products tend to be homogeneous with respect to composition. Microgel particles are usually synthesized by emulsion polymerization or copolymerization at elevated temperature using rapidly stirred solutions in order to obtain stable dispersions [16]. It was recently shown that the microgels and nanogels can be obtained by addition or step-growth polymerization of the polyfunctional monomers in solution. The aqueous dispersions of the PEO-*cl*-PEI particles were prepared by dissolving of lyophilized powder of the copolymer in the deionised water or buffer solution followed by sonication for 3–4 min. It is well recognized that a balance between the osmotic pressure and the polymer elasticity sets the physical dimensions of a hydrogel microsphere [39]. The osmotic pressure results from the net difference in concentration of mobile ions between the interior of the microgel particle and exterior solution. For ionic polymer gels fixed charged groups attract hydrated counter ions, which tend to expand the gel. Interaction of the nanogel with the surfactant involves Interaction between polyions and oppositely charged surfactants is a cooperative process in which the ionic head-groups of the surfactant bind to the polyion repeating units while the surfactant alkyl groups segregate into hydrophobic domains . Binding of various anionic surfactants with cationic polyamine segments of PEO-*cl*-PEI networks was studied by fluorescence probe (pyrene) technique [24]. This process characterized by a 'critical association concentration' (CAC), indicating the onset of surfactant binding to the polyion chains of network. Formulations of PEO-cl-PEI nanogels with oligonucleotides involves Addition of oligonucleotide to PEO-cl-PEI nanogel dispersion at pH 7.0 resulted in immediate formation of polyelectrolyte complexes due to the binding of oligonucleotide molecules to the PEI chain fragments of the nanogel [41]. To allow for the targeted delivery of nanogels in the body the surface of the nanogel particles can be modified with various bio specific ligands. Various coupling strategies can be used for this purpose including covalent attachment of the ligand moiety to the free amino groups of the PEI fragments in PEO-*cl*-PEI nanogel. One simple way to introduce various ligands in nanogel particles consists in the partial modification of PEI fragments with biotin moieties [41]. Biotin *p*-nitrophenyl ester was used for modification of amino groups of PEO-cl-PEI. The amount of biotin moieties conjugated to PEO-*cl*-PEI was determined using analytical assay based on the competitive displacement of a fluorescent dye, 2-anilinonaphthalene-6-sulfonic acid, in the reaction with avidin.

Uptake of oligonucleotide-loaded nanogel within the cells involves in that positively charged nanoscale particles are believed to bind electrostatically with the negatively charged cell membranes, which is followed by the internalization of these particles within the cells through the adsorptive endocytosis. The study of the internalization of the oligonucleotide-loaded PEO-*cl*-PEI nanogel suggested that the positively charged particles (N/P ratio 8) are much more efficiently taken up within the cells than the electroneutral particles (N/P ratio 4) [42]. Therefore, to enhance the uptake of the loaded nanogel particles these particles were coupled with insulin and transferrin using the biotin-avidin coupling technique. As a result a drastic enhancement of accumulation of the particles within the cells was observed. In order to evaluate the possibility of using PEO-cl-PEI nanogel for oral administration of oligonucleotides, the permeability studies were carried out using polarized Caco-2 cell monolayer's and standard Side-Bi-Side two-chamber device as an in vitro model of gastrointestinal epithelium [42]. The transfer of the free or nanogel-incorporated oligonucleotide in the receiver chamber was assayed using a fluorescence technique.

Thermosensitive sol-gel reversible hydrogels

A particularly interesting and important polymeric system is hydrogel forming solutions by a simple phase transition (sol–gel transition) in water without any chemical reaction or external stimulation. This system provides simplicity and safety in *in vivo* situations. The sol phase is defined as a flowing fluid, whereas the gel phase is non-flowing on an experimental time scale, while maintaining its integrity. Natural biopolymer gels have been used as food and food processing aids as well as in pharmacy. Now, discuss some of the biopolymer used forms gels in water. These are agarose, cellulose derivatives, carrageenan, Gellan, amylase, amylopectin.

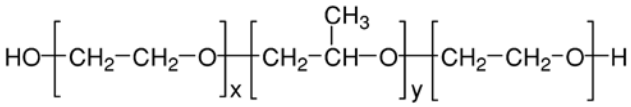
Most natural polymers form a gel phase on lowering the temperature. However, aqueous solutions of some cellulose derivatives exhibit reverse thermogelation (gelation at elevated temperatures). An interesting reverse thermogelation of a combination of chitosan and glycerol phosphate disodium salt was reported by Chenite *et al.* [43] all those below mentioned polymers re used in the hydrogel preparation.

(a) N-Isopropylacrylamide copolymer

N-Isopropylacrylamide homopolymer (poly-(NiPAAM) and its copolymers are discovered for the structure–property relationship, drug delivery, tissue engineering and also for enzyme or protein modification. Below the LCST, this is mostly contributed by the hydrogen bonding between polymer polar groups and water molecules, leads to dissolution of the polymer. Above the LCST, the entropy term (hydrophobic interactions) dominates, causing precipitation of the polymer in water. Gelation was attributed to polymer chain entanglements and the weak physical association of polymer precipitates with fewer ionisable groups at lower temperatures while maintaining hydration by more charged and expanded polymer strands. Isolated islets of Langerhans suspended in the polymer solution were effectively entrapped in the gel when the solution temperature was raised from 25^{0}C to body temperature, and the gel showed no cytotoxicity. Another advantage of the gel was the significantly higher permeability of insulin secreted from entrapped islets, because of the gel's heterogeneous character, rather than a traditional cell-entrapping matrix of alginate [20].

(b) PEG/PPO block copolymer

The commercial poly (ethylene oxide-b-propylene oxide-b-ethylene oxide)

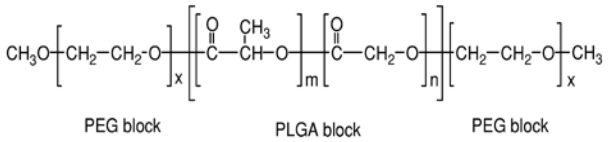


Poloxamers (ICI)) series with various molecular weights and PEG/PPO block ratios was used as a non-ionic surfactant, and the aqueous solutions of some Poloxamers exhibited phase transitions from sol to gel (low temperature sol–gel boundary) and from gel to sol (high temperature gel–sol boundary) as the temperature in creased monotonically when the polymer concentration was above a critical value. Recently, mechanistic studies on the phase transition and characterization of the solution and gel states of Poloxamers were reported using various instrumental techniques, such as ultrasound velocity, dynamic and static light scattering small angle neutron scattering (SANS) and microcalorimetry [43].

Taken together, triblock copolymers form micelles which equilibrate with Poloxamer unimers at low temperature above the critical micelle concentration (CMC). As the temperature increases, the equilibrium shifts from unimers to spherical micelles, reducing the number of unassociated unimers in solution, leading to an increase in the micelle volume fraction. Polymer coupled with Poloxamers also exhibit gelation in water [44]. This unique sol-to-gel transition has made the system attractive as an injectable drug delivery matrix in an in situ gel-forming drug depot. Most applications are based on Poloxamer PF-127 and include delivery of protein / peptide drugs, such as insulin, urease, interleukin-2, epidermal growth factor bone morphogenic protein (BMP), fibrobroblastic growth factor (FGF), and endothelial cell growth factor (ECGF). Some low-molecular-weight Poloxamers are classified as inactive ingredients for currently marketed drug products [45]. For example, Poloxamer 188, PEO–PPO–PEO (3500–1570–3500), is used as an intravenous injection formulation and an oral formulation, and Poloxamer 407, PEO–PPO-PEO (4300–3770–4300), as an ophthalmic solution. Because of the dissociation of packed micelles in an excess of water, the gel integrity of Poloxamers does not persist for more than a few days. In vitro experiments showed that 25 wt% of Poloxamer 407 gel was completely dissolved in the release medium in 4 h. For 35 wt% Poloxamer 407, the gel was 50% dissolved in 4 h [46]. Therefore, Poloxamer formulations are only useful for a short period after administration.

(c) PEG/PLGA block copolymers

Poly(ethylene glycol-b-L lactic acid-b-ethylene glycol) (PEG–PLLA–PEG) was synthesized by ring-opening polymerization of L-lactide onto monomethoxy poly(ethylene glycol) (MW 5000), which produced PEG–PLLA diblock copolymers, followed by coupling of the resulting diblock copolymers with hexamethylene diisocyanate to produce triblock copolymers with a PLLA central block (MW 2000–5000).



The mechanism of the sol-to-gel transition (lower transition) of an aqueous solution of a PEG–PLGA–PEG triblock copolymer is believed to be a micellar expansion accompanying an increase in aggregation number driven by hydrophobic forces [47]. Aqueous Poloxamer solutions are known to undergo the sol-to-gel transition by a shift in equilibrium from unimer to micelle, whereas the PEG–PLGA–PEG triblock copolymer in water seems to undergo the sol-to-gel transition by micellar growth. On increasing the PEG length of a PEG–PLGA–PEG triblock copolymer from 550 to 780 at a fixed PLGA length of 2300, the phase diagrams were shifted to higher temperatures (D*T* | 18^{0}C). The gel region remained almost constant. The gel strength is mainly determined by the hydrophobic block. The DL-lactic acid moiety is more hydrophobic than glycolic acid in PLGA. A similar interpretation can be drawn regarding the effect of the PLGA length on the sol–gel transition. Block copolymers have been investigated intensively for the solubilisation and stabilization of water-insoluble drugs, such as cyclosporin A and paclitaxel, and various protein pharmaceuticals, including Zn-insulin, porcine growth hormone, and glycosylated granulocyte colony stimulating factor (G-CSF) [48].

Characteristic of the Hydrogels

Polymer molecules consist of small molecular units, the so-called monomers, which can be arranged in a sequence to form a long polymer chain or to form branched polymer molecules with side chains. Generally, all polymers are solvophilic to certain solvents. Not cross-linked polymers are soluble in presence of these solvents. Due to the interconnections between the polymer chains cross-linked polymers are insoluble but swell by solvent absorption. If they can swell in water they are called hydrogels. In behavior of hydrogels in solvents unlike "normal" solvophilic polymers stimuli-responsive hydrogels exhibit a first-order- or a continuous (also called second-order) phase transition behavior. They exhibit two phases. A separated phase of the gel is dominated by polymer-polymer interactions. In this case the gel reaches its maximal value of hydrophobicity and shrinks. The second phase, a mixed phase, is characterized by solvent-polymer-interactions, which aspire the best mixing of polymer and aqueous solution.

Special solvent-responsive hydrogels can be additionally temperature-sensitive. Such gels have a slightly hydrophobic nature and contain groups, which preferably interact with water molecules by hydrogen bonds which cause the hydrogel swelling. These hydrogen bonds depend on the temperature [49]. In polyelectrolyte hydrogels, contains weak acidic and weak basic groups, respectively, which can be ionized. For example, gels containing acidic groups are deprotonated in basic surrounding conditions as following:

$$[RCOOH]_{gel} + [OH^-]_{aq} \rightarrow [RCOO^-]_{gel} + H2O.$$

Therefore, the density of likewise charged groups within the network strongly increases accompanied by an adequate generation of mobile counter ions inside the gel, which induces the phase transition due to electrostatic repulsion. In an acidic ambient the acidic gel protonated

$$(RCOO^-)_{gel} + (H^+)_{aq} \rightarrow (RCOOH)_{gel}$$

This resulting in a decrease of both the charge density and the content of mobile counter ions within the hydrogel leading to gel shrinking [49]. The swelling and shrinking of hydrogels requires a transport of matter, which is time-consuming. To initiate a volume phase transition two transport mechanisms have to be considered.

First, the initiating stimulus has to be transferred into the hydrogel, such as temperature difference, solvents or ions, which is associated with a change of the balance of the osmotic pressure. The transport occurs either energetically by heat transfer (described by the thermal transfer coefficient, D_T) or by continuous mass diffusion of a solvent into the hydrogel (described by the spontaneous mass transfer coefficient, D_S). Then as a second mechanism, to obtain the swelling equilibrium of the changed osmotic pressure balance, the hydrogel swells or shrinks absorbing or releasing swelling agent.

Sensor Transducers in hydrogels

Sensor transducers are components, which convert the non-electrical changes of properties of the stimuli-responsive hydrogel into an evaluable signal, in most cases an electrical signal. Two basic principles can be used in gel sensors:

1. Transducers based on mechanical work performed by hydrogel swelling and shrinking.

2. Transducers observing changes in properties (e.g. densities, mass, volume, stiffness) of free swelling gels.

CONCLUSION

The fantastic properties of hydrogels suggest that they will have numerous future applications as the next generation materials for biological and biomedical applications. Furthermore, fundamental research of hydrogel behaviour also contributes significantly in understanding of the biological functions of various biomolecules. There have been lot of studies on hydrogels that exhibit changes in swelling in response to physicochemical stimuli, such as pH, temperature, etc. Currently, biomolecule-sensitive hydrogels that respond to specific biomolecules, such nonionic gel,as saccharides and proteins, had gaining a huge importance, since saccharides and proteins are useful as markers to monitor several physiological changes as well as for site-specific drug delivery. It has been reported in many researches that molecular interactions, such as lectin–glucose, phenylboronic temperature-induced phase transitions over a wide range of acid–glucose, and antigen–antibody interactions, can provide the tools for creating biomolecule-sensitive hydrogels for the development of self-regulated drug delivery systems. Combining the functions of biomolecules, such as enzymes, with pH or temperature-sensitive polymers can also lead to construction of biomolecule-sensitive systems. Still most of biomolecule-sensitive hydrogels require further research; they are likely to explore as important biomaterials in the near future. Few of the studies discussed in this paper will definitely lead to a better understanding of the structures and functions of hydrogels as well as promising strategies for the development of novel Bio-sensitive hydrogels.

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