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HYDROGELS FROM SOFT CONTACT LENSES AND IMPLANTS TO SELF-ASSEMBLED NANOMATERIALS

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Abstract

Hydrogels were the first biomaterials designed for clinical use. Their discovery and applications as soft contact lenses and implants are presented. This early hydrogel research served as a foundation for the expansion of biomedical polymers research into new directions: design of stimuli sensitive hydrogels that abruptly change their properties upon application of an external stimulus (pH, temperature, solvent, electrical field, biorecognition) and hydrogels as carriers for the delivery of drugs, peptides, and proteins. Finally, pathways to self-assembly of block and graft copolymers into hydrogels of precise 3D structures are introduced.

Keywords

Hydrogels; soft contact lenses; biocompatibility; stimuli-responsive materials; drug delivery; self-assembly of block and graft copolymers; hybrid hydrogels

INTRODUCTION

Hydrogels are water-swollen polymeric materials that maintain a distinct three-dimensional structure. Their classification1 may be based on the source: natural, synthetic, or hybrid hydrogels (composed of synthetic and natural molecules); nature of the crosslinking: covalent or physical gels; nature of the network: homopolymer, copolymer, interpenetrating, or double networks; physical structure: homogeneous (optically transparent), microporous, and macroporous hydrogels, and on their fate in the organism: degradable and non-degradable hydrogels.

Due to their high water content, most hydrogel structures possess excellent biocompatibility. The amount of water in the equilibrium-swollen state is a balance between the thermodynamic force of mixing (hydration) and the retractive force of the three-dimensional network. The mixing force depends mainly on the hydrophilicity of the polymer backbone (characterized by the interaction parameter, χ, polymer-solvent), the retractive force on the number of crosslinks connecting polymer chains into a three-dimensional network.2 Consequently, there is a wide variety of design options for the preparation of hydrogels of different structures and properties. The traditional methods of hydrogel synthesis were limited in the control of their detailed structure, but novel approaches based on genetic engineering and hybrid hydrogels, have considerably enhanced this research. These techniques permit the insertion of biorecognition moieties into the structure of macromolecules that influence the self-assembly into precisely defined three-dimensional structures. As a result, the application potential of hydrogels, in addition to traditional areas such as biomaterials and drug delivery systems, has expanded to other fields, such as microfluidics and nanotechnology.

The review is focused mainly on hydrogel discovery and early developments along with my views on the future of hydrogel research. A vast amount of work could not be covered, but it has been discussed in other, numerous reviews.[1,3–13] I apologize that, due to space limitations, it was not possible to mention contributions of all scientists to the field in the last 50 years.

DISCOVERY

In the early 1950s Otto Wichterle and Drahoslav Lím from the Prague Institute of Chemical Technology (both soon moved to the Institute of Macromolecular Chemistry (IMC), Czechoslovak Academy of Sciences, Prague) initiated a research program to design polymers for medical use. Some commodity polymers had been applied in humans previously, but this was the first attempt to design polymers for human use with properties to fulfill criteria of biocompatibility. The target was the design of new biomaterials for applications in ophthalmology. The main features of their design were (included in their grant proposal in 1952) were: a) shape stability and softness similar to that of the soft surrounding tissue; b) chemical and biochemical stability; c) absence of extractables; and d) high permeability for water-soluble nutrients and metabolites across the biomaterial tissue-interface. It is amazing that these hypotheses are still valid for soft contact lenses. Only when hydrogels are used as implants is hypothesis d) not operative; it is known that implants are encapsulated with a fibrous collagen capsule; the latter is the rate controlling diffusion barrier across the interface.

Based on this rationale, Lím started efforts to synthesize new hydrogels. First, he considered polymerization of *N*-vinylpyrrolidone.[14] However, it was not available, so Lim's first experiments focused on partially methacryloylated polyvinylalcohol optionally containing –CO-NH_2 groups and on partially methacryolylated mannit.[15] Polyvinylalcohol was chosen due to its previous use in human implants (Ivalon[16]). Methacryloyl esters were chosen because the structure of the polymer reflects a pivalic (trimethylacetic) acid structure. The latter was known to be stable to pure hydrolysis and no similar structure in the nature was known, making enzymatically catalyzed hydrolysis less probable. The polyvinylalcohol route produced optically clear hydrogels containing 80–90% water[15] but these hydrogels did not show mechanical properties necessary for use in contact lenses.

One year later, Lím was studying polymers of triethyleneglycol dimethacrylate for applications in fiberglass laminates, when he serendipitously identified a novel hydrogel material. He was synthesizing the triethyleneglycol dimethacrylate monomer by acid catalyzed transesterification of methyl methacrylate with triethylene glycol. At the end of the reaction, the work-up comprised neutralization of the acid, dilution with water to isolate the water-insoluble triethyleneglycol dimethacrylate, washing the organic layer with water, drying and isolating the pure product by distillation. One day Lím had to catch the train to his home, so he stopped the reaction early, but managed to add water to separate the layers before leaving. In the morning, he noticed that the water layer turned into a clear hydrogel overnight. Obviously, it was a copolymer of triethyleneglycol monomethacrylate with triethyleneglycol dimethacrylate.[14] Detailed evaluation and comparison of crosslinked methacrylate esters of triethyleneglycol, diethyleneglycol, and ethyleneglycol led to the final selection of a hydrogel for soft contact lenses - copolymer of 2-hydroxyethyl methacrylate (HEMA = ethyleneglycol monomethacrylate) with ethylene dimethacrylate (EDMA = ethyleneglycol dimethacrylate). These polymers were described in a seminal paper published in Nature in 1960.[17] Crosslinked HEMA was a fortunate choice for two reasons: first, it showed proper hydrophilic/hydrophobic balance and produced hydrogels with suitable mechanical properties for soft contact lens applications; second, its optical properties changed only slightly with changes in the concentration of crosslinking agent. At

that time, the preparations of HEMA contained varying (small) amounts of crosslinker, EDMA. Due to the relatively high χ parameter of the system polyHEMA – water,18 the equilibrium swelling degree of HEMA hydrogels (about 40% of water in the equilibrium swollen state) changed only marginally with minor changes of the concentration of the crosslinking agent in the polymerization feed. This allowed for production of soft contact lenses with reproducible properties even if the quality of the monomers varied to a certain extent.

Wichterle and Lím initiated biocompatibility studies of hydrogels; these first results were published in 195919 and 1960.20,21 Wichterle then collaborated with local companies (Dental, later Dioptra) to produce soft contact lenses. These experiments resulted in soft (hydrogel-based) lenses possessing excellent optical properties in the central part of lenses but with irregular edges. The collaboration with Dr. M. Černý from the Dioptra Company resulted in 1958 in soft contact lenses with acceptable edges, which minimally irritated the eye.22 These were the first samples that were administered by Dr. M. Dreifus in 1959 to patients at the Second Ophthalmologic Clinic in Prague. The tests have proven the ability of soft contact lenses to correct eyesight and be well tolerated by the patients.22 Finally, Wichterle prepared in 1961 the soft (hydrogel-based) contact lenses by the spin casting process23,24 using a constructor set of his grandson (Fig. 1). The use of centrifugal monomeric casting in rotating open molds produced soft contact lenses of amazing quality. 25,26 In 1963 Wichterle developed another way of producing of soft contact lenses which utilized lathing machines. The glass transition temperature of polyHEMA is above 100 °C. 27 Consequently, crosslinked HEMA polymerized in bulk (“xerogel”) is a hard material that can be cut into blocks and worked up on a lathing machine similarly to the production of “hard” contact lenses from poly(methyl methacrylate). After the final shape is produced, the xerogel is placed into an aqueous environment and a soft contact lens with predetermined optical properties is formed. In 1964 a small US company, National Patent Development Corporation (NPDC), expressed interest in the new technology and organized a lecture tour in the USA for Wichterle and Dreifus.22 Soon after, NPDC bought the license covering soft contact lenses from the Czechoslovak Academy of Sciences and in 1966 sub-licensed it to Bausch & Lomb. This initiated the soft contact lens industry in the USA. Further evolution of soft contact lens polymers has been reviewed elsewhere.28,29

EARLY DEVELOPMENTS IN HYDROGEL RESEARCH

Research on the design of hydrogels suitable for numerous medical applications was initiated at the same time as the soft contact lens work. In ophthalmology, glaucoma microcapillary drains,30 fillings for the restoration of detached retina,31 and fillings after enucleation21 were studied. Hydrogels have also been designed for augmenting vocal cords, 32 preventing scar formation after surgery, and as coverings for perforated ear drums.33 These applications were the driving force behind a detailed study of the relationship between the physical and chemical structure of crosslinked hydrophilic polymers and their biocompatibility (tissue reaction, calcification).34–39 These results were translated into clinical applications: one successful example was the use of HEMA-based hydrogels in rhinoplasty (Fig. 2), which produced long-term biocompatibility and excellent cosmetic results.40

On the scientific side, persistent research activities of several IMC laboratories on different aspects of hydrogel synthesis, structure, and properties provided the foundation of the science of three-dimensional systems in general and hydrogels in particular. Initial studies concentrated on hydrophilic (glycol, diglycol, and triglycol) esters of methacrylic acid; the kinetics of linear and crosslinking polymerizations of these monomers were evaluated. 18,41–43 The mechanism of the crosslinking copolymerization revealed the importance of

the structure of the crosslinking agent on the ratio of crosslinking, cyclization, and formation of pendent vinyl groups.41 The mechanical and swelling properties of hydrogels,44–48 diffusion in hydrogels,49 and optical properties of hydrogels50 were assessed in detail.

While hydrophilic esters of methacrylic acid18,36–38,41–43 produced crosslinked polymers (hydrogels) with excellent characteristics from the application point of view, the *N*-substituted amides of (meth)acrylic acid represented a group of polymers whose properties could be easily manipulated by choosing the proper substituent on the amide nitrogen.34,35,39,51–53 Moreover, a crystalline monomer, *N*-(2-hydroxypropyl)methacrylamide (HPMA), was synthesized51,54,55 which ensured a reproducible synthesis of water-soluble polymeric carriers of drugs.56 Esters of (meth)acrylic acid of similar hydrophilicity always contained a small (variable) amount of diester as an impurity. This was a minor problem for the synthesis of hydrogels, but prevented the synthesis of water-soluble polymers with reproducible molecular weight distribution. Structures of early hydrogels are shown in Figure 3.

Research in the IMC Prague stimulated many researchers worldwide to work on the subject. An important event, which motivated numerous young scientists worldwide to get involved in hydrogel research was the ACS symposium organized by J. Andrade in 1975.4 Ratner and Hoffman published an excellent overview of chemical structures and biomedical applications of hydrogels up to 1975 as one of the chapters in the Proceedings.5

BIOCOMPATIBILITY OF HYDROGELS

Biological processes that follow implantation66–68 depend on the chemical structure, physical structure (porosity), and surface microarchitecture of hydrogels. A systematic study of the biocompatibility of hydrogels34–39 revealed no significant differences in the healing-in of hydrogels of different chemical compositions based on esters and/or *N*-substituted amides of (meth)acrylic acid. However, major differences have been observed for hydrogels with different morphology.37,38

Hydrogels prepared by crosslinking copolymerization of HEMA with EGDMA are an excellent model for the study of the relationship between porosity and biocompatibility. Due to the fact, that the interaction parameter (χ) polymer-water for this system is 0.7–0.8 (depending on crosslinking density),18 phase separation may occur during copolymerization, which depends on the amount of water in the feed. Manipulating the water to monomer ratio in the feed permits the formation of homogeneous (transparent) hydrogels (<50% water in the feed), microporous hydrogels (50–70% water in the feed), and macroporous spongy hydrogels with interconnecting channels (>70% water in the feed).38 Thus, the biocompatibility of hydrogels with identical chemical structure, but differing in porosity could be compared.37,38 The implantation of both homogeneous and heterogeneous hydrogels resulted in fibrous capsule formation. However, following implantation of porous hydrogels, in contrast to homogeneous hydrogels, newly formed blood capillaries and an eosinophilically stained exudate, penetrated into the implant. The intensity and the area of penetration were greater with higher hydrogel porosity. An investigation of calcium deposits using von Kóssa staining revealed the dependence of the extent and localization of calcium deposits on porosity. There was only sporadic calcification in the margin of the implant following implantation of homogeneous or microporous hydrogels; however, with an increase in porosity, calcification occurred. The site of the deposition moved from the margin of the implant toward its center with increasing porosity.38 Early studies on the biocompatibility of hydrogels have been summarized in ref.39

These results were corroborated in clinical settings. Implantation of homogeneous HEMA-based hydrogels to treat nasal malformation resulted in minor calcification at the margin of the implant (about 50% of patients evaluated after 3–10 years). Apparently, with scalpel damaged surface (due to surgeons modifying the size of the hydrogel implants in the operation room) connective tissue accumulated and initiated calcium deposition. Minor calcium deposition did not affect the biocompatibility or the final cosmetic effect.40

STIMULI-SENSITIVE HYDROGELS

Inspired by early works of Kuhn, Katchalsky et al. on crosslinked polyelectrolytes and the possibility of transferring chemical energy into mechanical work,69,70 the research focus shifted to the design of stimuli-sensitive hydrogels. These materials show varying degrees of response (continuous or discontinuous changes in swelling) to minor changes in environmental conditions, such as pH, temperature, ionic strength, quality of solvent, or biorecognition.71

Dušek and Patterson72 theoretically predicted in 1968 that changes in external conditions might result in abrupt changes in the hydrogel degree of swelling (phase transition). Indeed, ten years later Tanaka et al.73 and others59,74 verified the theory by experimental observations.

The first manipulation of hydrogel structure to produce pH-sensitive hydrogels was published by Kopeček's laboratory in 1971.75 Introduction of ionogenic groups into HEMA hydrogels (crosslinked with EDMA) permitted control of their permeability75 and specific resistance57 as a function of pH. Hydrogel membranes containing methacrylic acid (MA) units possessed higher permeability of NaCl in alkaline conditions, whereas membranes containing *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA) units showed increased permeability under acidic conditions. Permeability of ampholytic membranes, containing both MA and DMAEMA units passed through a minimum at the isoelectric point, and increased as the pH deviated from the isoelectric point in either direction (Fig. 4). The results for the relationship between the structure of hydrogel membranes and specific resistance revealed similar trends.57

Temperature-sensitive hydrogels are usually based on polymers exhibiting lower critical solution temperature (LCST), i.e., the gels collapse as temperature increases. Apparently, below the LCST, water molecules form hydrogen bonds with polar groups on the polymer backbone and organize around hydrophobic groups as iceberg water. Above the LCST, bound water molecules are released to the bulk with a large gain in entropy resulting in collapse of the polymer network. Allan Hoffman76 and Sung Wan Kim77 initiated the studies of poly(*N*-isopropylacrylamide) (PNIPAAm) and NIPAAm copolymers for developing drug delivery matrices.76–78 Poly(*N*,*N*-diethylacrylamide)59,78 and its copolymers with *N*-*tert*-butylacrylamide demonstrated similar behavior. The sensitivity of NIPAAm-based hydrogels to other stimuli, e.g., electric current,79 light,74,80 salt,81 and chemical stimuli,82 has also been demonstrated and studied in detail. One successful strategy to increase the response dynamics of stimuli-sensitive hydrogels was the introduction of short grafts. Okano's comb-type grafted NIPAAm hydrogels demonstrated rapid de-swelling responses to temperature changes.83

HYDROGELS IN DRUG DELIVERY

Hydrogels were studied as antibiotics and anticancer drug delivery depots soon after their discovery.84–87 Initial studies concentrated on polyHEMA, with later studies focused on hydrogels based on HEMA copolymers,88 polyacrylamide,89 N-vinylpyrrolidone copolymers,90 and polyvinylalcohol.91 HEMA hydrogels were studied as matrices for

protein delivery.92 Different chemical structures have been tailor-made to match the physiological need. For example, HPMA copolymer based hydrogels were synthesized with entrapped anticancer drugs93,94 or containing degradable oligopeptide crosslinks and the drug (DOX) bound via an degradable oligopeptide spacer.95 Various natural polysaccharides were used for drug delivery;96,97 polyalginate hydrogels were designed to permit combination delivery of anticancer drugs with different release profiles.98

Crosslinking hydrogels with compounds that contain an aromatic azobond endows them with colon-specific degradability. Due to a low proteolytic activity in the colon, these hydrogels are being developed for protein delivery. For example, copolymers of *N*,*N*-dimethylacrylamide with acrylic acid (to introduce pH-sensitivity), *N*-tert.-butylacrylamide (to improve mechanical properties), and a crosslinking agent such as 4,4′-di(methacryloylamino)azobenzene are suitable for colon delivery of proteins.65,99–103 Following oral administration, the hydrogel delivery system reaches first the stomach; at the low pH, the hydrogels have a low equilibrium degree of swelling and thus may protect the peptide or protein drug against digestion by proteolytic enzymes. As the gels pass down the GI tract, swelling increases due to ionization of carboxylic acid groups in response to pH increase. Finally, in the colon, the aromatic azobonds are reduced, the hydrogel disintegrates and the protein is released into an environment of low proteolytic activity. Dextran hydrogels are also suitable as colon-specific drug delivery systems, due to microbial dextranase activity in the colon.104

An example of a combination of stimuli-sensitivity and drug delivery are hydrogel-based mimics of secretory granules containing the anticancer drug doxorubicin (DOX).105 A microgel prepared by copolymerization of methacrylic acid with methylene-bis-acrylamide was loaded with DOX and then coated with a phospholipid bilayer. The hydrogel microspheres exhibited pH- and ion-dependent volume phase transitions and were suitable for triggered DOX release.

Extensive studies are underway to understand the effect of hydrogel structure on controlling the osteogenic or chondrogenic differentiation of human mesenchymal or embryonic stem cells.106–109 Hydrogels that mimic the natural extracellular matrix are another important aspect of bioresponsive hydrogels design. These hydrogels are degradable by cell-excreted matrix metalloproteases (MMP); the aim is to design hydrogels that can control cellular invasion and movement.110,111 The use of PEG-based hydrogels in these applications has been described in an excellent review.112

HYDROGELS WITH ENHANCED MECHANICAL PROPERTIES

Major use of hydrogels has occurred in applications where mechanical properties are not decisive, such as soft contact lenses,29 electrophoresis,113 implants in soft tissue,40 and tissue engineering.114 Numerous strategies have been employed to improve mechanical properties of hydrogels by copolymerization,115–117 optimizing crosslinking density,118 or using composite hydrogel materials.119–120 Reasonable improvements have been achieved. However, three approaches resulted in dramatic improvements in mechanical properties of hydrogels:

Double network hydrogels

Gong and Osada designed double network (DN) hydrogels,121,122 a subset of interpenetrating networks composed of two hydrogel structures, one a highly crosslinked polyelectrolyte, the other a loosely crosslinked or uncrosslinked neutral hydrogel. For example, a DN composed of two mechanically weak hydrophilic networks, poly(2-acrylamido-2-methylpropanesulfonic acid) and polyacrylamide, results in a DN with

outstanding mechanical properties. Hydrogels containing about 90% water possessed an elastic modulus of 0.3 MPa and fracture stress of ~10 MPa, demonstrating both hardness and toughness. This was explained by effective relaxation of locally applied stress and dissipation of crack energy through a combination of the two networks with different structure and densities.121⁻123

Hydrogels containing sliding crosslinking agents

Okumura and Ito chemically crosslinked two cyclodextrin molecules, each threaded on a different PEG chain (end-capped with a bulky group, such as adamantan), and produced a sliding double ring crosslinking agent.124 Crosslinked gels (Fig. 5) possess freely movable (sliding mode) crosslinks,125,126 which resulted in outstanding mechanical properties – a high degree of swelling in water and a high stretching ratio without fracture. The sliding double ring crosslinks in these topological gels apparently equalized the tension along the polymer chains ("pulley effect").123,124

Nanocomposite hydrogels

Haraguchi et al. designed novel clay-containing hydrogels based on *N*-isopropylacrylamide (NIPAAm) using hectorite, $[Mg_{5.34}Li_{0.66}Si_8O_{20}(OH)_4]Na_{0.66}$, as a multifunctional crosslinker.127 Compared to traditional chemically crosslinked NIPAAm hydrogels, the mechanical properties of these unique organic-inorganic hydrogels were considerably enhanced.128 The tensile moduli and tensile strength were found to be proportional to clay content.129 Similar hydrogels were prepared from *N*,*N*-dimethylacrylamide and clay. Up to 1500% elongation-at-break values were obtained.130 It is interesting to note that mechanically robust hydrogels could only be prepared by radical polymerization of NIPAAm in the presence of clay. Mixing of polyNIPAAm with clay did not produce homogeneous hydrogels with robust mechanical properties.131

LIMITATIONS OF TRADITIONAL HYDROGELS

Numerous monomers and crosslinking agents have been used for the synthesis of hydrogels with a wide range of chemical compositions (Fig. 3).

Traditional methods of hydrogel synthesis (Fig. 6), i.e. crosslinking copolymerization of monovinylic and multivinylic compounds,18,41⁻43,51,57,65,99,132,133 crosslinking of polymer precursors,100,134 polymer-polymer reaction,101,102 or combination of two processes103 provided limited control of their three-dimensional structure. As a demonstration, we consider the copolymerization of a vinylic and a divinylic compound, such as diethyleneglycol monomethacrylate with diethyleneglycol dimethacrylate.41 The following events contribute to the limited control and to the extent of the reactions of the pendent vinyl group (crosslinking, cyclization, unreacted pendent vinyl groups):

a. *Structure of the crosslinking agent*. The distance between vinyl groups influences the amount of cyclization. Using crosslinking (divinylic) agents with different distances between two vinyl groups the efficiency of crosslinking varied according to the probability to form cycles. Assuming that the second vinyl of the crosslinking agent attaches to the growing polymer chain immediately after the first one (alternating intermolecular-intramolecular chain propagation), ethyleneglycol dimethacrylate (EGDMA) forms a 9-membered ring, diethyleneglycol dimethacrylate (DEGDMA) a 12-membered ring, and triethyleneglycol dimethacrylate (TEGDMA) a 15-membered ring. Indeed, EGDMA was two times more effective crosslinking agent than DEGDMA and TEGDMA in agreement with the low probability of 9-membered ring formation (Fig. 7). This is not

surprising when considering that divinylic compounds capable of forming 5- or 6-membered rings cyclopolymerize into soluble products.135

b. *Difference in hydrophilicity of monomer and crosslinking agent*. When copolymerizing a hydrophilic monomer (e.g. HEMA) with a hydrophobic crosslinking agent (e.g. EGDMA), the formation of first crosslinks creates hydrophobic centers. Partitioning of crosslinking agent into these domains results in hydrogels with inhomogeneous distribution of crosslinks (see, e.g. ref.136).

c. *Diffusion control of termination, crosslinking, and eventually propagation*. The increase in viscosity with conversion results in the diffusion control of termination once the movement of macromolecule becomes hindered. This results in an imbalance between the rate of termination and rate of initiation with continuous increase in the concentration of active species (radicals) and concomitant increase of the rate of polymerization (Trommsdorff effect).41 Diffusion control of crosslinking and propagation may take place at higher conversions. These effects amplify with increasing concentration of crosslinking agent in the feed.

d. *Glass transition temperature*. When copolymerizing in bulk (or low amount of solvent) glass transition temperature (T_G) may be reached during the polymerization. The reaction will stop at the conversion where the mixture of the produced polymer and remaining monomer has a T_G equal to the polymerization temperature.137

e. *Amount and character of solvent*. The amount of solvent (concentration of monomers in the feed) has an impact on the efficiency of cyclization described above. The lower the concentration of monomers, the higher the probability of cycle formation. The character of solvent has an impact on chain conformation during the crosslinking copolymerization. In addition, phase separation may occur during crosslinking copolymerization resulting in formation of porous hydrogels. A typical example is the copolymerization of HEMA with EGDMA in water at feed monomer concentrations < 45%.1،38

f. *Conversion*. There is an exponential relationship between the concentration of crosslinks and conversion in a vinyl-divinyl copolymerization. To ensure batch-to-batch reproducibility, polymerization conditions need to be selected to ensure that the polymerization proceeds to full conversion.

NOVEL DIRECTIONS IN HYDROGEL RESEARCH

Controlled chemistry

There were numerous attempts to control the polymerization process for hydrogel synthesis. Early studies from Andrade's group focused on the synthesis of stereoregular HEMA-based hydrogels.138 It was shown that the swelling degree in water depended on the stereoregularity. With the advancement of living radical polymerization (LRP) techniques, atom transfer radical polymerization139 and reverse addition-fragmentation chain transfer (RAFT) polymerization140 the control of the molecular weight distribution of primary chains in the hydrogel was achieved. The application of (copper catalyzed) Huisgen cycloaddition of azides and terminal alkynes ("click" chemistry)141،142 (Fig. 8) and of peptide ligation reactions143 in the synthesis of hydrogels further contributed to the design of well-defined materials. Anseth and coworkers have shown that by combination of photopolymerization with click chemistry it is possible to spatially pattern biomolecules within PEG hydrogels.144 Introduction of photodegradable bonds into hydrogels permits post-gelation manipulation of gel properties in situ.145 The wide applications of these modern approaches will further improve the control of hydrogel synthesis and 3D structure.

Self-assembled hydrogels

Hydrogels may self-assemble from block and graft copolymers driven by hydrophobic interactions between the blocks.146 Triblock copolymers, PEG-PLGA-PEG form in-situ hydrogels suitable for drug delivery.147,148 An interesting approach for hydrogel self-assembly which involves a higher degree of biorecognition, is crosslinking by stereocomplex formation. Hennink et al. grafted dextran with either L- or D-lactic acid oligomers.149 Mixing of two graft copolymers results in hydrogel formation. These gels are suitable for protein delivery and are fully degradable in vivo.150 Finally, the design of copolymers, whose self-assembly into hydrogels is mediated by recognition motifs found in nature, such as coiled-coils or β-sheets, enhances the potential for the formation of precisely defined three-dimensional structures.

The highest degree of biorecognition might be achieved by employing natural motifs. Self-assembly of natural polymeric building blocks often results in supramolecular biological structures, which have several characteristic features. First, the assembly process is highly specific and mediated by molecular recognition. Second, it is stimuli-responsive; external signals, such as temperature and pH, can trigger or terminate assembly.151,152 Finally, the assembly and disassembly processes are typically reversible, allowing precise and dynamic control of the structures and their properties.

These approaches were used in hydrogel design. Theoretically, this method provides spontaneous formation of precise 3D structures with predetermined properties. Examples of genetically engineered polymers as well as hybrid systems containing such systems are shown below to demonstrate the potential of this new design paradigm.

Biorecognition motifs—Both peptide/protein and DNA motifs have been used in hydrogel design. However, we shall focus on systems in which the coiled-coil153 and β-sheet forming peptides or enzyme mutants were combined with synthetic macromolecules to produce responsive hydrogels.

Coiled-coils: The coiled-coil, a supercoil formed by two or more strands of α-helices, will be used as an example to demonstrate the potential for the design of well-organized hydrogel structures. The primary sequence of a typical coiled-coil is composed of 7-residue repeats, designated as heptads. The amino acid residues in a heptad are conventionally denoted as “a, b, c, d, e, f, g”. Hydrophobic residues at positions “a” and “d” form an inter-helical hydrophobic core, providing a stabilizing interface between the helices. Charged residues at positions “e” and “g” form electrostatic interactions, which contribute to coiled-coil stability and mediate specific association among helices (Fig. 9). The versatility of this motif, especially the possibility to manipulate its stability and specificity by modifying the primary structure (up to 10^{-15} M stabilities may be achieved154), bodes well for the successful design of a new class of hydrogel biomaterials.

Beta-sheet forming peptides: Beta-sheets are important structural elements in proteins (Fig. 9). β-Strands are aligned adjacent to each other and are stabilized by hydrogen bonds between the carbonyl oxygen of an amino acid in one strand and the backbone nitrogen of a second amino acid in another strand. The strands (at least two, but frequently more) can arrange in parallel or antiparallel fashion to form the β-sheets.

Self-assembly of genetically engineered triblock copolymers—Tirrell’s pioneering work demonstrated that ABA block copolymers, where block A is a coiled-coil forming peptide and block B a random coil, self-assemble into hydrogels.155 The self-assembly occurs as a balance between the oligomerization of the helical ends and swelling

of the central water-soluble polyelectrolyte segment. Temperature and/or pH-responsiveness may be achieved by manipulating the amino acid sequence of the coiled-coil domains. Minor modifications in their structure have a strong impact on the stimuli-sensitivity of self-assembled hydrogels. For example, the thermal stability of the coiled-coil containing proteins can be manipulated in a predictable way by substituting amino acids in the coiled-coil domain (Fig. 10). An important observation was that these structures were reversible after denaturation.156,157 Changes in environmental conditions (temperature, pH, removal of denaturing agent by dialysis) result in refolding of the α-helical structure. Experimental data clearly indicate that manipulation of the structure of the coiled-coil sequence in triblock copolymers, composed of two coiled-coil blocks flanking a random coil, permits rational design of reversible, stimuli-sensitive hydrogels.156 Similar behavior of triblock copolymers was observed during 2D self-assembly on a solid subtrate.158

Hybrid hydrogels

Hydrogel systems that possess components from at least two distinct classes of molecules, for example, synthetic polymers and biological macromolecules, are usually referred to as hybrid hydrogels. Conjugation of peptide domains and synthetic polymers may lead to novel materials with properties superior to those of individual components. Compared to synthetic polymers, proteins and protein modules have well-defined and homogeneous structures, consistent mechanical properties, and cooperative folding/unfolding transitions. The peptide domain may impose a level of control over the structure formation at the nanometer level; the synthetic part may contribute to the biocompatibility of the hybrid material. The synergistic combination of two types of structures may produce new materials that possess novel properties and unprecedented levels of structural organization.159–161 In principle, the responsiveness of hydrogels may be directly related to the defined structure of the protein crosslinks. By optimizing the amino acid sequence, responsive hybrid hydrogels tailor-made for a specific application may be designed.

Crosslinking of polymer precursors with genetically engineered protein domains—A new strategy of hybrid hydrogel synthesis entails the non-covalent attachment of genetically engineered coiled-coil protein motifs to hydrophilic synthetic HPMA copolymer backbone. The physical crosslinking was established by self-assembly of the coiled-coil domains.151,162 A temperature-induced hydrogel collapse was observed that corresponded to the structural transition of the coiled-coil domains from an elongated helix to an unfolded state. Hydrogels formed by crosslinking of HPMA copolymer precursors with coiled-coil modules underwent dramatic volume transitions (de-swelling up to 10-fold) at the melting temperature of the coiled-coil modules.151 This is a new temperature response mechanism for hydrogels that can be tuned over a wide temperature range by assembling gels with coiled-coils that have different melting temperatures.

Hydrogels self-assembled from graft copolymers via formation of coiled-coil antiparallel heterodimers—Recently, self-assembly of graft copolymers into hybrid hydrogels was demonstrated.163,164 A novel hybrid hydrogel system based on HPMA copolymers consisting of a hydrophilic polymer backbone and a pair of oppositely charged peptide grafts was characterized.163 Two distinct pentaheptad peptides (CCE and CCK) were designed to create a dimerization motif and serve as physical crosslinkers. Consequently, the graft copolymers, CCE-P (P is the HPMA copolymer backbone) and CCK-P, self-assembled into hybrid hydrogels; the process was modulated by the formation of antiparallel heterodimeric coiled-coils. The use of graft copolymers provides the advantage of decreased steric hindrance of the polymer backbone due to the “in-register” alignment of the peptide grafts. Equimolar mixtures of the graft copolymers, CCE-P/CCK-P, have been observed to self-assemble into hydrogels in PBS (phosphate buffer) solution at

neutral pH at concentrations as low as 0.1 wt.% (Fig. 11). The kinetics of self-assembly can be followed by dynamic light scattering.164 The formation of these hybrid hydrogels was reversible.163

Hydrogels self-assembled from HPMA copolymers containing β-sheet forming peptide blocks—The self-assembly of HPMA hybrid diblock165 and graft166 copolymers into hydrogels mediated by β-sheet peptide blocks was recently evaluated. Circular dichroism and Congo Red binding studies showed that the peptide block imposed its β-sheet structural arrangement on the structure of diblock copolymers.165 A new hybrid hydrogel based on polyHPMA grafted with a β-sheet peptide, Beta11, was designed. CD spectroscopy indicated that folding ability of β-sheet peptide was retained in the hybrid system, whereas the sensitivity of the peptide towards temperature and pH variations was hindered. The morphology of the hydrogels was characterized by long-range order with minimal lateral aggregation.166

Hybrid hydrogels containing proteins—Whole proteins and/or their mutants have been used in hybrid hydrogel design. Park developed hybrid hydrogels containing concanavalin A capable of *sensing glucose* levels.167,168 Free glucose competes for the concanavalin A (ConA) binding sites in poly(acrylamide-co-allyl glucose) physically crosslinked by Con A, leading to disruption of the physical crosslinks with a concomitant increase in swelling.

Miyata et al. designed *antigen-responsive hydrogels*169 as a semi-interpenetrating network (IPN) composed of a soluble poly[acrylamide-co-acryloyl rabbit IgG (antibody)] embedded in a hydrogel prepared by copolymerization of acrylamide, methylene-bis-acrylamide and acryloyl-goat-anti rabbit IgG (antigen). The resulting semi-IPN was sensitive to the presence of free antigen. Binding of free antigen resulted in the decrease of crosslinking density with a concomitant increase in the equilibrium degree of swelling.169 Lu et al. synthesized antigen-sensitive hydrogels by copolymerization of *N*-isopropylacrylamide, polymerizable antibody Fab‘ fragment from monoclonal anti-fluorescein BDC1 antibody, and *N*,*N*‘-methylene-bis-acrylamide.170 When the hydrogel was alternatively exposed to fluorescein and polyamidoamine dendrimer-fluorescein, significant reversible volume changes were observed.

Enzymatic activity of a protein, creatinine kinase, within a hydrogel exhibits an autonomous self-beating motion in an ATP solution. This is a result of a periodical change in Ca++ concentration with concomitant changes in crosslinking density.171

Volume changes in hybrid hydrogels triggered by conformational changes in proteins: Calmodulin was incorporated into acrylamide-based172 or poly(ethylene glycol)-based173,174 hybrid hydrogels. Upon binding its ligand (Ca^{++}, phenothiazine), calmodulin changes conformation resulting in alterations in hydrogel’s degree of swelling. Murphy et al. 174 modified calmodulin (T34C, T110C) double mutant with PEG diacrylate to produce a protein with two polymerizable groups. The latter was photocrosslinked into a trifluoperazine-sensitive hydrogel.

Yuan et al. designed dynamic hybrid hydrogels capable of *translating enzyme-substrate recognition into mechanical motion*.175 The design principle was based on incorporating controlled motion of macromolecules into three-dimensional materials. Synthetic HPMA copolymers with pendant maleimide groups were chosen as the backbone. To create two attachment points, a triple mutant of adenylate kinase, AKtm (C77S, A55C, V169C), was designed and engineered. The distance between C^{α}-atoms of the residues 55 and 169 decreases from 29.5 Å in the apo-enzyme to 12.4 Å when forming the enzyme-substrate

(ATP) complex. The AKtm was incorporated into hydrogels through maleimide-thiol reaction. When the hydrogel was exposed to substrate (ATP) a volume change occurred. The degree of deswelling increased with increasing concentration of the substrate. The volume changes were reversible. Similar results were obtained when 4-arm PEG with maleimide groups at chain termini was crosslinked with AKtm.175 The uniqueness of the design is the combination of biorecognition with a catalyzed chemical reaction – the transfer of a phosphate group. There are numerous enzymes that undergo conformational changes following the binding of a substrate in the active site. Thus, this is a new paradigm for hybrid hydrogel design (Fig. 12), where a variety of chemical reactions can be combined with biorecognition and to transform nano-scale conformational changes into macroscopic motion.

CONCLUSIONS AND FUTURE PROSPECTS

Hydrogels were the first biomaterials designed for clinical use. Numerous applications of traditional hydrogels have proven their biocompatibility and functional properties. New approaches to improve mechanical properties of hydrogels have contributed to a better understanding of structural factors important for hydrogel properties and expanded their application potential.

Future research in hydrogels will concentrate on the design of 3D structures with programmed biofunctionality. There is a critical need to study major factors involved in self-assembly of hybrid hydrogels and to establish structural and physicochemical criteria for the formation of reproducible, reversible 3D hydrogel networks with precisely defined structures and properties.

All scientific evidence seems to indicate that basic and translational research in hydrogels has great potential.176,177 Numerous new designs, e.g. involving protein domains containing non-canonical amino acids,178 successful attempts to control the morphology of self-assembling peptide fibers,179 artificial glycoproteins for controlling cell responses,180 hydrogels as matrices for stem cell renewal,181 hydrogels as the building material for microchemotaxis devices,182 sensors,183 valves in microfluidic devices,172,184,185 enhanced use of DNA recognition motifs,186–188 and improved synthetic methods189 demonstrate the versatility of the hybrid/smart hydrogel approach.

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Biography

Jindřich (Henry) Kopeček, received his Ph.D. in Macromolecular Chemistry from the Institute of Macromolecular Chemistry (IMC) and D.Sc. in Chemistry from the Czechoslovak Academy of Sciences, Prague, Czech Republic. His postdoctoral studies were done at the National Research Council of Canada. Before joining the University of Utah in 1986, he was Head of the Laboratory of Biodegradable Polymers at the IMC. He is currently Distinguished Professor of Pharmaceutical Chemistry and Distinguished Professor of Bioengineering at the University of Utah. He served as President of the Controlled Release Society (1995–1996), Chairman of the Department of Pharmaceutics and Pharmaceutical Chemistry (1999–2004), and Chair of the Biomaterials and Interfaces Study Section at the National Institutes of Health (2003–2006). His awards include the Millennial Pharmaceutical Scientist Award (2000), Paul Dawson Biotechnology Award (2001), Heyrovský Medal of the Academy of Sciences of the Czech Republic (2003), Distinguished International Scientist Award of the Japanese Biomaterials Society (2006), and Honorary Professorship, Sichuan University, China (2007). Kopeček's research interests are focused

on biorecognition of macromolecules, bioconjugate chemistry, drug delivery systems, and self-assembled biomaterials. Hydrogels from his laboratory have been in clinical use and HPMA copolymer - anticancer drug conjugates in clinical trials. His publications have been cited over 10,000 times.

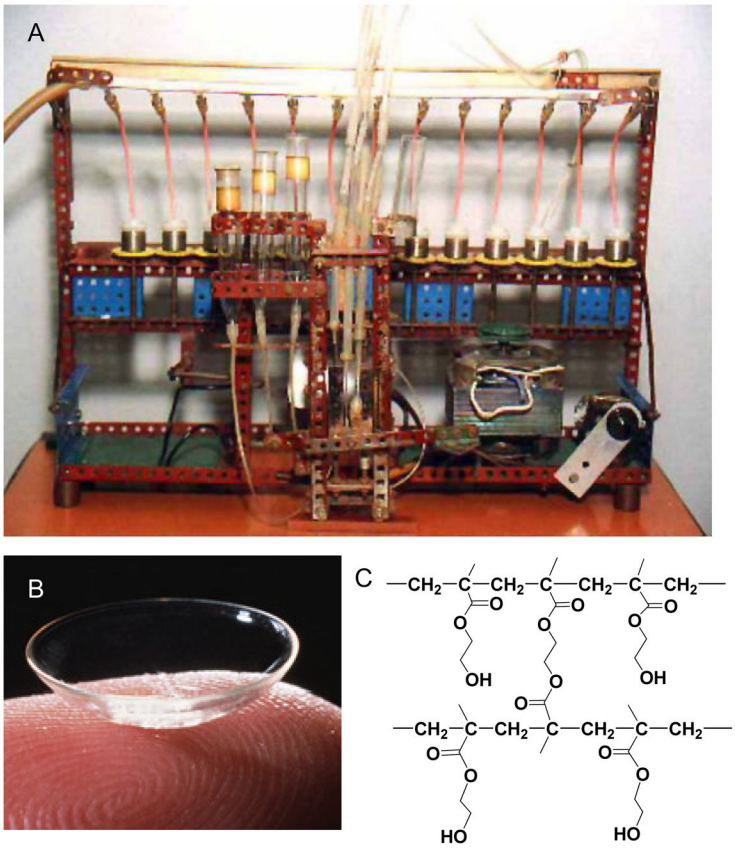


Figure 1.

A) First polymerization apparatus for spin casting of soft contact lenses built from a construction set of O. Wichterle's grandson (www.21stoleti.cz/view.php?cisloclanku=2005021823); B) contact lens (www.soft-contacts.biz); C) Structure of hydrogels used in early soft contact lenses – copolymer of HEMA with EDMA.

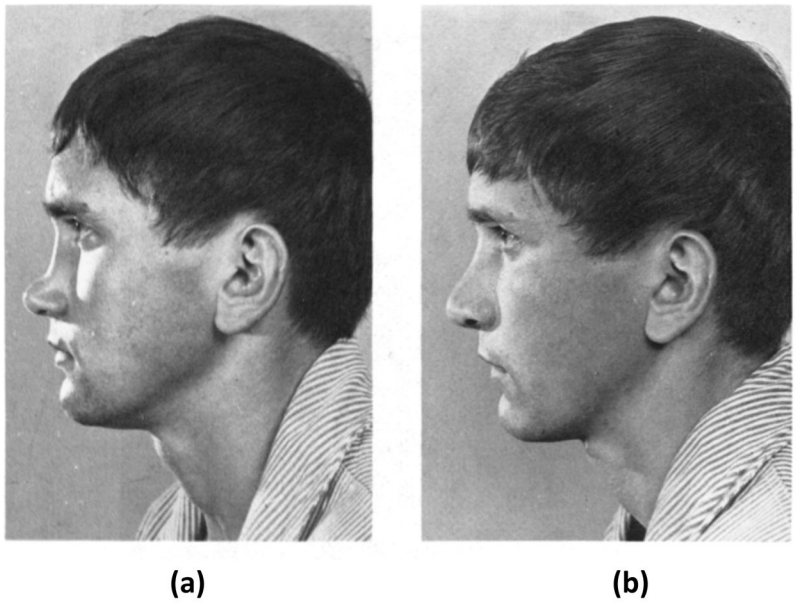


Figure 2.
Hydrogel implants. Clinical use of hydrogels (copolymers of HEMA with EDMA) in rhinoplasty. (a) patient before surgery; (b) patient after surgery.40

Early Hydrogel Structures

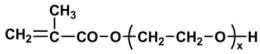
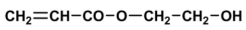
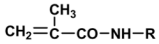
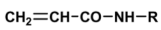
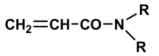
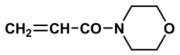
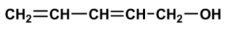
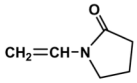
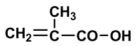
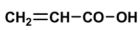
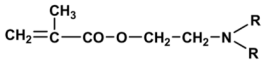
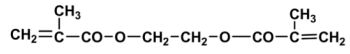
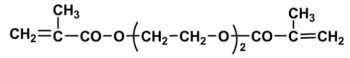
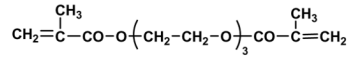
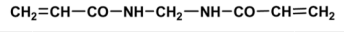
Monomers		References
	hydrophilic esters of methacrylic acid	17, 18, 37, 38, 41-43
	2-hydroxyethyl acrylate	57
	*N*-alkylmethacrylamides	34, 39, 58
	*N*-alkylacrylamides	35, 39
	*N,N*-dialkylacrylamides	39, 59
	*N*-acryloylmorpholine	35, 60
	2,4-pentadienol	61, 62
	N-vinylpyrrolidone	63
Comonomers		
	methacrylic acid	36, 64
	acrylic acid	57, 65
	*N, N*-(dialkylamino)ethyl methacrylate R=CH_3, CH_2CH_3	36
Crosslinking agents		
	ethylene dimethacrylate	
	diethyleneglycol dimethacrylate	
	triethyleneglycol dimethacrylate	
	*N,N'*-methylenediacrylamide	

Figure 3.
Structures of early hydrogels.

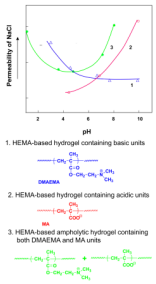


Figure 4.

Stimuli sensitive hydrogels. First detailed study of the relationship between the structure of hydrogels and their pH-dependent properties. pH sensitivity of 2-hydroxyethyl methacrylate copolymer-based hydrogels was achieved by introducing ionogenic groups into the hydrogel structure. The permeability of NaCl through a hydrogel membrane containing basic units of *N*,*N*-dimethylaminoethylmethacrylate (1) increased in the acidic region, whereas the permeability of membranes containing methacrylic acid units (2) increased in the alkaline region. Permeability through an ampholytic membrane (3), containing approximately the same quantities of methacrylic acid and *N*,*N*-dimethylaminoethylmethacrylate, passed through a minimum in the isoelectric point and increased as the pH diverged from the isoelectric point in either direction. Adapted from reference 75.

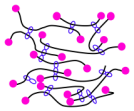


Figure 5.
Mechanically improved hydrogels. Schematic structure of a topological sliding hydrogel. α-Cyclodextrin moieties threaded on PEG chains (end-capped with a bulky group) were crosslinked by trichlorotriazine producing double (and probably triple) ring crosslinks freely movable along the PEG chains. The sliding of crosslinks results in a decrease of inhomogeneities of the network and in regulation of the tension with concomitant enhancement of mechanical properties. Adapted from references 124–126.

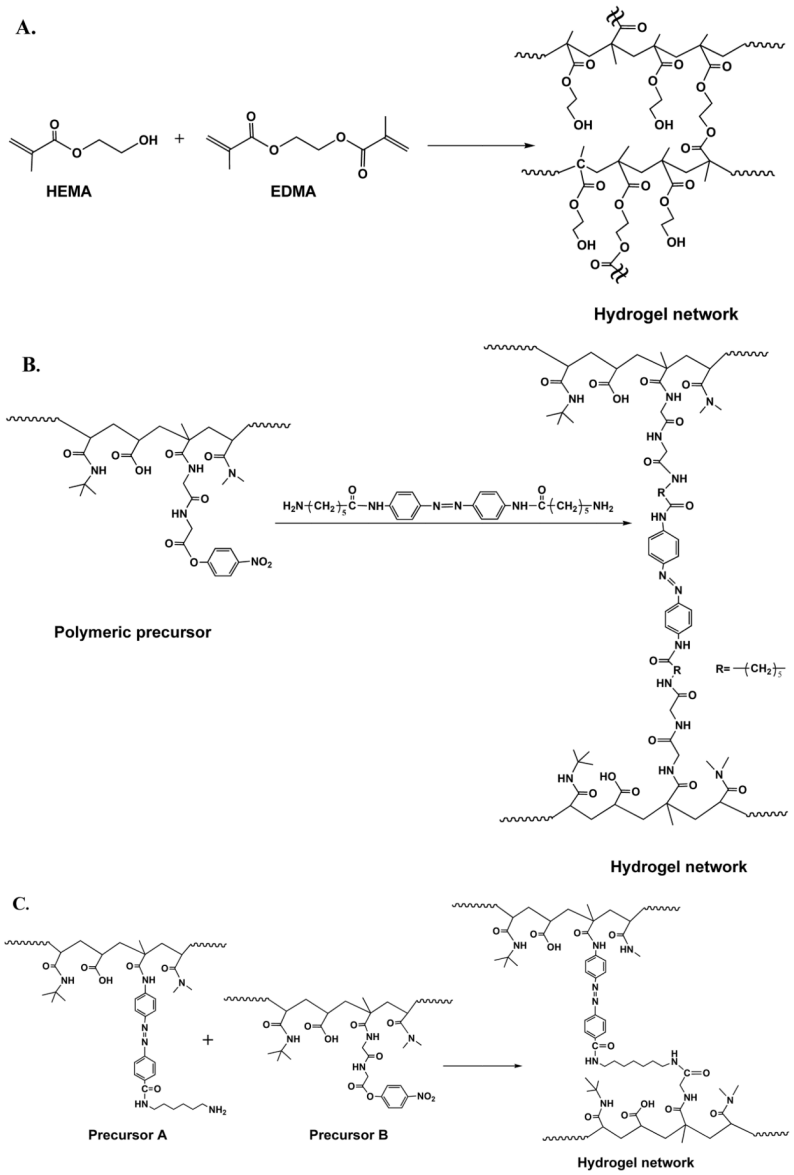


Figure 6.
Traditional hydrogel synthesis. A) Crosslinking copolymerization;18 B) Crosslinking of polymer precursors with a low-molecular-weight crosslinking agent;100 C) Polymer-polymer reaction of two polymer precursors.102

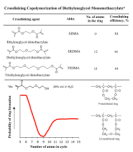


Figure 7.
The impact of the distance between the vinyl groups of a crosslinking agent on the crosslinking efficacy is related to the probability of ring formation by alternating intermolecular-intramolecular chain propagation.41

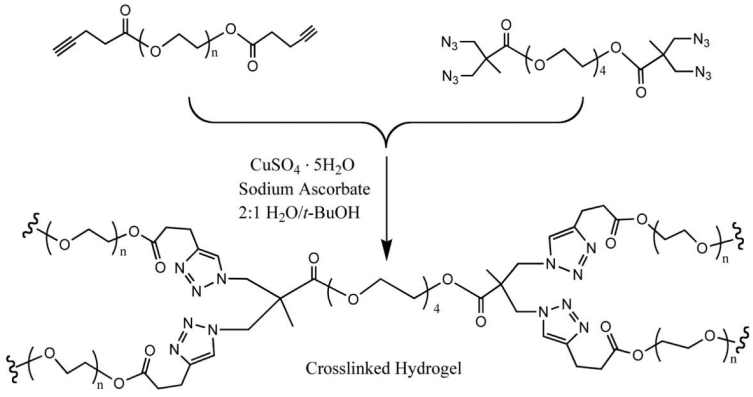


Figure 8.
Hydrogel synthesis based on click chemistry and PEG-based building blocks.141

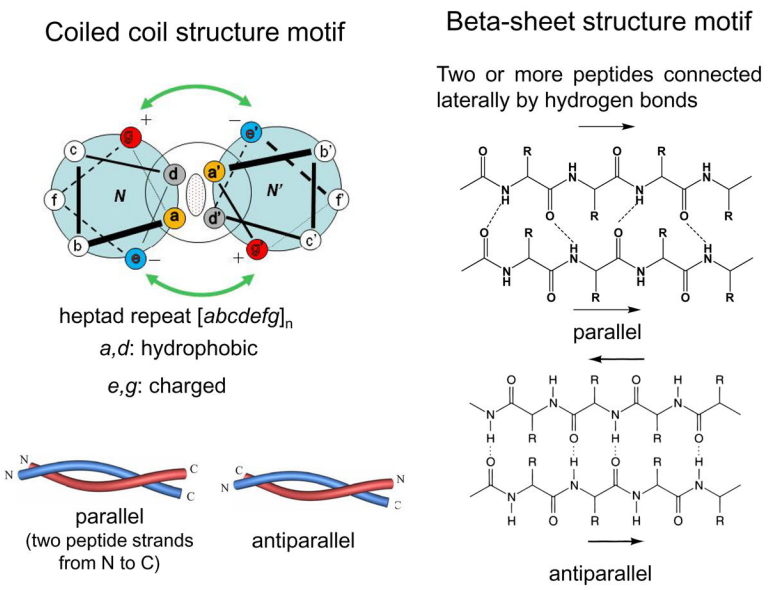


Figure 9.
Peptide motifs used in the design of hybrid hydrogels: Left panel: Coiled-coils; Right panel:β-sheets.

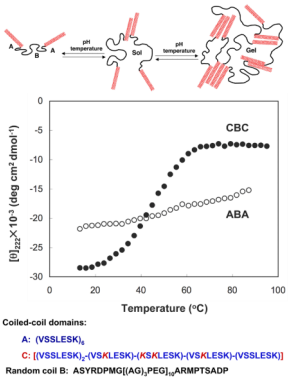


Figure 10.

Self-assembly of genetically engineered ABA and CBC triblock copolymers into hybrid hydrogels. Minor manipulation of the primary structure of coiled-coil forming blocks provides a tool to modify temperature responsiveness of the 3D construct. Four amino acid residues of block A were replaced with lysine to produce block C.156

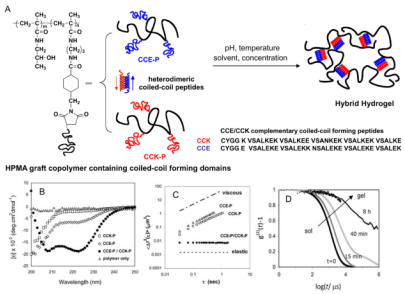


Figure 11.

Self-assembly of graft copolymers into hybrid hydrogels mediated by antiparallel heterodimer coiled-coil formation. Two distinct pentaheptad peptides (CCE and CCK) were designed to create a dimerization motif and serve as physical crosslinkers. Equimolar mixtures of the graft copolymers, CCE-P/CCK-P (P is the HPMA copolymer backbone), self-assemble into hydrogels in PBS (phosphate buffer) solution at neutral pH at low concentrations. A) Structure of copolymers and schematic of hydrogel formation through antiparallel heterodimeric coiled-coil association;163 B) CD spectra (in PBS; 100 μM of peptide) of CCE-P, CCK-P, and their equimolar mixture CCE-P/CCK-P;163 C) Comparison of gelation behavior among individual HPMA copolymer conjugates, CCE-P and CCK-P, and their equimolar mixture by microrheology;163 D) Normalized intensity autocorrelation functions for solutions of equimolar mixture of CCE-P and CCK-P (10 mg/mL) at time intervals indicated.164

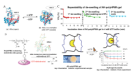


Figure 12.
Hydrogels containing a triple mutant of adenylate kinase, AKtm (C77S, A55C, V169C), as a crosslinker are able to translate the enzyme conformation change upon binding a substrate into mechanical motion.175 A) Ribbon diagram of the structure of adenylate kinase (AKe) in two conformational states: open state (a); and closed state (b). B) HPMA-based hydrogels crosslinked with AKtm and dithiothreitol (DTT) and the macroscopic motion of hydrogels triggered by substrate recognition. The deswelling ratios of gel containing variable amounts of AKtm as crosslinker (0–100%). C) Three cycles of deswelling of hydrogel crosslinked with 100% of AKtm. Adapted from reference 175.