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Novel crosslinking methods to design hydrogels ☆

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ABSTRACT

Hydrogels are presently under investigation as matrices for the controlled release of bioactive molecules, in particular pharmaceutical proteins, and for the encapsulation of living cells. For these applications, it is often required that the gels degrade under physiological conditions. This means that the originally three-dimensional structure has to disintegrate preferably in harmless products to ensure a good biocompatibility of the hydrogel. In this overview, different chemical and physical crosslinking methods used for the design of biodegradable hydrogels are summarized and discussed. Chemical crosslinking is a highly versatile method to create hydrogels with good mechanical stability. However, the crosslinking agents used are often toxic compounds, which have been extracted from the gels before they can be applied. Moreover, crosslinking agents can give unwanted reactions with the bioactive substances present in the hydrogel matrix. Such adverse effects are avoided with the use of physically crosslinked gels.

Contents

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1. Introduction

Hydrogels are polymeric networks, which absorb and retain large amounts of water. In the polymeric network hydrophilic groups or domains are present which are hydrated in an aqueous environment thereby creating the hydrogel structure. As the term 'network' implies, crosslinks have to be present to avoid dissolution of the hydrophilic polymer chains/segments into the aqueous phase. Hydrogels can also be described in a rheological way. Aqueous solutions of hydrophilic polymers at low or moderate concentrations, where no substantial entanglement of chains occurs, normally show Newtonian behavior. On the other hand, once crosslinks between the different polymer chains are introduced, the so obtained networks show visco-elastic and sometimes pure elastic behavior. Because of their water-absorbing capacity, hydrogels are not only subject of investigation of researchers interested in fundamental aspects of swollen polymeric networks, but have also found widespread application in different technological areas, e.g. as materials for contact lenses and protein separation, matrices for cell-encapsulation and devices for the controlled release of drugs and proteins. The reader is referred to a variety of excellent books and review papers that describe the fundamental aspects and application areas of hydrogels [1–9].

As mentioned, crosslinks have to be present in a hydrogel in order to prevent dissolution of the hydrophilic polymer chains in an aqueous environment. A great variety of methods to establish crosslinking has indeed been used to prepare hydrogels. Since it is advantageous for many applications that the hydrogels are biodegradable, labile bonds are frequently introduced in the gels. These bonds can be present either in the polymer backbone or in the crosslinks used to prepare the gel. The labile bonds can be broken under physiological conditions either enzymatically or chemically, in most of the cases by hydrolysis [9]. It is of course of great interest to have control over the degradation kinetics; in other words, it is important to have control over the parameters by which the degradation characteristics can be tailored. But degradability as such is not the ultimate solution. Once the hydrogels are implanted it is of the utmost importance that the gels have a good biocompatibility and that the degradation products formed have a low toxicity. This means that the compounds formed can either be metabolized into harmless products or can be excreted by the renal filtration process. In general, hydrogels possess a good biocompatibility. Their hydrophilic surface has a low interfacial free energy in contact with body fluids, which results in a low tendency for proteins and cells to adhere to these surfaces. Moreover, the soft and rubbery nature of hydrogels minimizes irritation to surrounding tissue [10–13]. The nature of the degradation products formed can be tailored by a rational and proper selection of the hydrogel building blocks.

In this contribution, novel crosslinking methods to design hydrogels are described and discussed. The characteristics and some potential applications of the gels are described in relation to their preparation methods. Both chemical and physical methods have been used to create hydrogels. In chemically crosslinked gels, covalent bonds are present between different polymer chains. In physically crosslinked gels, dissolution is prevented by physical interactions, which exist between different polymer chains. Both methods will be discussed in the next chapters.

2. Chemically crosslinked gels

2.1. Crosslinking by radical polymerization

Chemically crosslinked gels can be obtained by radical polymerization of low molecular weight monomers in the presence of crosslinking agents. Poly(2-hydroxyethyl methacrylate) (pHEMA) is a well known and frequently studied hydrogel system. This hydrogel was first described by Wichterle and Lim [14] and is obtained by polymerization of HEMA in the presence of a suitable crosslinking agent (e.g. ethylene glycol dimethacrylate). Using similar procedures, a great variety of other hydrogel systems has been synthesized [15]. The hydrogel characteristics, among which the swelling, can be modulated by the amount of crosslinker. Moreover, stimuli sensitive materials can be obtained by the addition of e.g. methacrylic acid (pH-sensitive gels [16]) or *N*-isopropylacrylamide (temperature-sensitive gels [17]). Besides by radical polymerization of mixtures of vinyl-monomers, chemically crosslinked hydrogels can also be obtained by radical polymerization of water-soluble polymers derivatized with polymerizable groups. Different water-soluble (synthetic, semi-synthetic and natural) polymers have been used for the design of hydrogels via this route. In particular dextran is used as a building block for (degradable) hydrogels. Dextran is a bacterial polysaccharide, which consists essentially of α-1,6 linked D-glucopyranose residues. The low molecular weight fractions of dextran (Mw between 40 and 100 kDa) have been used as a plasma expander [18] which has resulted in a good documentation of the pharmacological activities and side-effects of dextran. Dextran has therefore been investigated for the delivery of drugs, proteins and imaging agents [19]. Moreover, due to the presence of dextranase in the colon, dextran-based gels are under investigation as a colon delivery system [20]. Research on polymerizable dextran was pioneered by Edman et al. [21], who reacted dextran dissolved in water with glycidylacrylate (Fig. 1). A hydrogel was formed after the addition of an initiator system consisting of *N,N,N′N′*-tetramethylene-diamine and ammonium peroxydisulfate to an aqueous solution of the acryldextran also containing *N,N,*-methylenebisacrylamide. Enzymes were immobilized with almost full retention of their activity by an emulsion polymerization technique in microspheres of polyacryldextran [21,22]. Water-soluble polymers other than dextran, namely albumin [23], (hydroxyethyl)starch [24–26], poly-aspartamide [27–29], poly(vinyl alcohol) [30] and hyaluronic acid [31] were also derivatized with (meth) acrylic groups using essentially the method developed by Edman et al. Since the reaction is carried out in an aqueous solution, the degree of substitution is very low and difficult to control due to hydrolysis of glycidyl(meth)acrylate before and after reaction with the water-soluble polymer. Therefore, in our department an alternative method has been developed to synthesize methacrylated dextran [32]. In our approach, dextran is dissolved in a suitable aprotic solvent (DMSO) after which derivatization with glycidylmethacrylate (GMA), catalyzed by 4-(*N,N*-dimethylamino)pyridine, is carried out. Almost quantitative incorporation of GMA was found and the degree of substitution can be fully controlled. A detailed analysis by NMR [33] and by mass spectroscopy of the products obtained after enzymatic degradation [34] revealed that under the selected conditions the reaction of GMA and dextran was a transesterification resulting in a dextran derivative with the methacrylate group directly attached to the dextran chain (Fig. 1). The synthetic procedure developed was also suitable to derivatize other compounds with methacrylate groups, among which are inulin [35] and sucrose [36]. (Meth)acrylate groups can also be introduced in water-soluble polymers using (meth)acryloyl chloride [37,38], methacrylic anhydride [39], and by the subsequent reaction of dextran with bromoacetyl bromide and sodium acrylate [40]. Moreover, using enzymes as catalyst, (meth)acrylic groups have be introduced in mono- and di-saccharides, which can be used for the synthesis of hydrogels [41–43]. The synthesis is carried out in anhydrous pyridine and the products are normally obtained in a high yield (75%). In contrast to chemical methods, enzymatic synthesis results in a very good regioselectivity.

A polymerizable dextran derivative was obtained by reaction of dextran with maleic anhydride. These dextran derivatives can be converted into a hydrogel by UV-induced polymerization of the vinyl groups. The gels were not degradable under physiological conditions. They did, however, exhibit a strong pH-dependent swelling behavior due to the presence of carboxylic acid groups in the network [44]. Methacrylate groups coupled to water-soluble polymers are sensitive towards hydrolysis under physiological conditions [45].

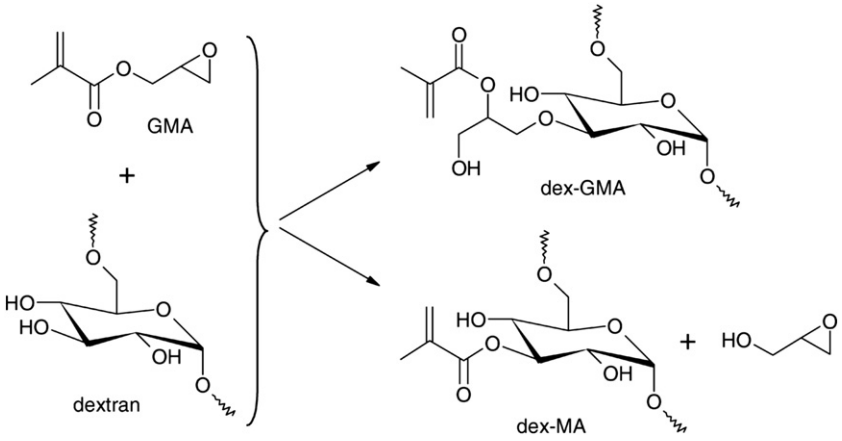

Fig. 1. Reaction of dextran with GMA.

However, after polymerization, the methacrylate esters are very resistant toward hydrolysis [46,47]. This means that gels derived from these polymers can only degrade under physiological conditions once the polymer main chains are hydrolyzed by a matching enzyme. This has been demonstrated for gels based on albumin [23], starch [25] and dextran [48]. Although methacrylated dextran could be degraded by dextranase, even at high degree of methacrylate substitution [34], it has been shown that for hydrogels derived hereof full degradation only occurred when the degree of methacrylate substitution (DS, number of methacrylate groups per 100 glucose units) was <4. For gels with an intermediate degree of substitution (DS between 4 and 7), partial degradation occurred whereas in gels with a high degree of substitution (DS>7) hardly any degradation occurred [48–50]. As an alternative for these enzymatically degradable gels, chemically degradable gels were designed. These gels were synthesized by connecting the polymerizable group and the water-soluble polymer via hydrolytically sensitive structures. Hubbell and colleagues synthesized macromers having a poly(ethylene glycol) central block, extended with oligomers of α-hydroxy acids and terminated with acrylate groups. By radical polymerization of the acrylate groups of the macromers a hydrogel is formed. Radicals were generated after exposure to UV light of an aqueous solution of the macromer to which a suitable photoinitiator (2,2-dimethoxy-2-phenylacetophenone) dissolved in a reactive solvent (*N*-vinylpyrrolidone) was added. These hydrogels were indeed biodegradable with PEG, lactic acid (or other α-hydroxy acids, depending on the macromer) and oligo(acrylic acid) as degradation products. The degradation time varied from 1 day to 4 months and could be tailored by the choice of macromer, especially by the choice of degradable link [51]. In a recent study of Metters et al., it was demonstrated that the degradation could be accelerated by copolymerization of PEG–PLA macromers with acrylic acid [52]. The UV-curable PEG–PLA–acrylate gels were used as protein releasing matrices [53–55]. The permeability of the hydrogel can be altered by changing the composition of the macromer. In our department, we synthesized methacrylated dextran derivatives in which the polymerizable groups are connected via (a) hydrolytically sensitive group(s) to a dextran backbone. The hydrolyzable groups are either a carbonate ester in dex-HEMA or a combination of a carbonate ester and lactic acid groups (dex-lactate-HEMA) (Fig. 2, Ref. [56]). After polymerization of these derivatives the gels degraded under physiological conditions due to the presence of (carbonate) ester groups in the crosslinks, yielding dextran, lactic acid and short fragments of pHEMA as degradation products (schematically shown in Fig. 3). The degradation time varied from 1 day to more than 3 months and can be controlled by the type of ester group in the crosslinks, the crosslink density of the gel, and the length of the lactic acid spacer [47,57]. Interestingly, the gels had a good biocompatibility both in the form of implants and in the form of injectable microspheres, and the degradation time in vivo (after subcutaneous implantation in rats) was around the same as found in vitro [58–60]. This means that also in vivo the degradation is most likely caused by chemical hydrolysis. These degradable dextran hydrogels are suitable systems for the controlled release of pharmaceutical proteins [47,61]. Protein loaded injectable microspheres can be prepared in an all aqueous system [62]. Since the protein is present during the hydrogel formation, it can be entrapped in pores of the gel which are smaller than the protein. The release of protein from these microspheres is then fully controlled by the degradation rate of the hydrogel matrix [63]. Recently, we even demonstrated that liposomes could be entrapped in dextran microspheres

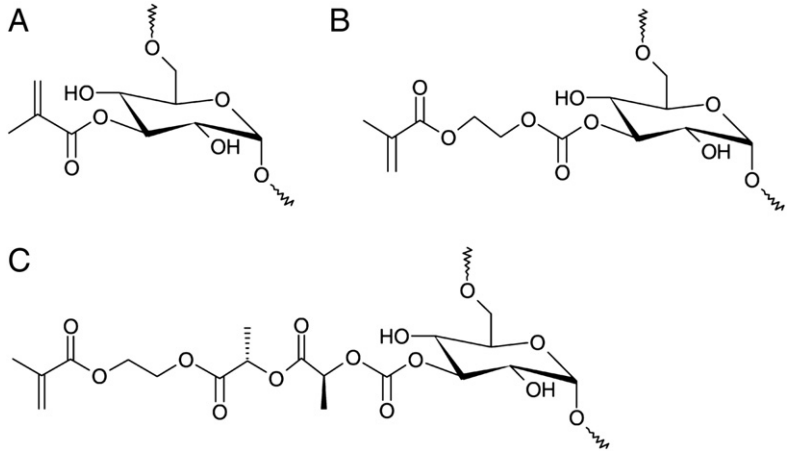

Fig. 2. Structures of (A) dex-MA, (B) dex-HEMA and (C) dex-(lactate)$_2$-HEMA.

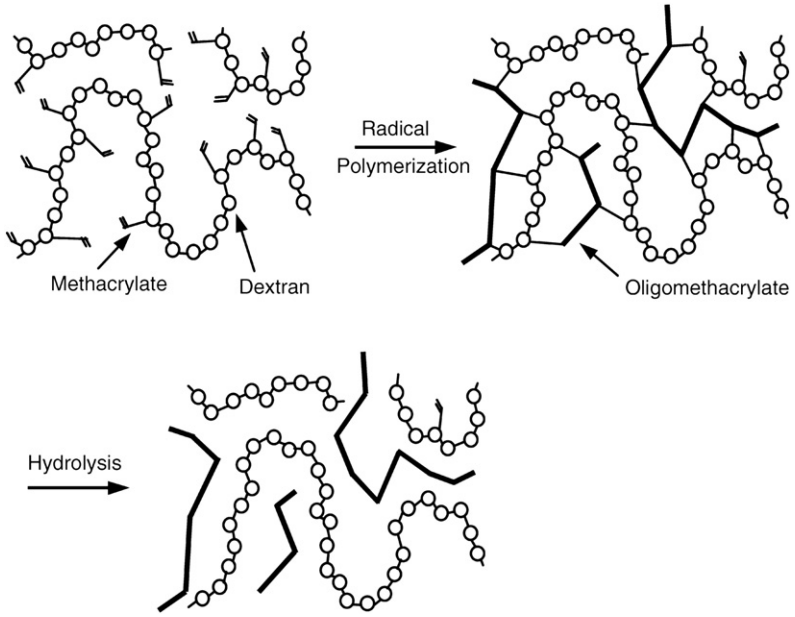

Fig. 3. Schematic representation of the formation and degradation (for dex-HEMA and dex-(lactate)$_2$-HEMA) of dextran hydrogels. Gels are formed by radical polymerization of the methacrylate groups using KPS and TEMED as initiator system. Degradation occurs by hydrolysis of the carbonate and lactate ester for dex-HEMA and dex-(lactate)$_2$-HEMA, respectively.

and released in their intact form [64]. Another route to dextran-based hydrogels was published by Zhang et al. They modified dextran with a polymerizable group, either acryloyl chloride [65] or allyl isocyanate [66]. The dextran derivatives were dissolved in DMF together with a poly(D,L-lactic acid) diacrylate macromer (PDLLAM). A network was obtained by UV-induced polymerization. The swelling of the gels depended among others on the ratio of dextran/PDLLA in the network, the degree of substitution of dextran with the polymerizable group and the UV-irradiation time [65]. These gels were investigated as a matrix for the release of albumin. Its release was dependent on the gel composition and was governed by a combination of diffusion and degradation of the matrix [67]. Although Zhang et al. claim that these hydrogels are biodegradable, complete biodegradability was not observed and is unlikely due to the choice of dextran derivatives.

The polymerization of the vinyl derivatized water-soluble polymers can be carried out with an initiator system composed of a peroxydisulfate and *N,N,N′N′*-tetramethylene-diamine (TEMED). This is a very efficient system, which gives rapid formation of the gel [32,68] even under mild conditions (room temperature, pH 7). However, unreacted peroxydisulfate and TEMED as well as their degradation products have to be extracted from the gel before in vivo application. Moreover, this initiator system can also damage proteins once they are present during preparation of the gels. In particular, methionine residues of protein can be oxidized [69]. However, the biological activity of oxidized IL-2 was almost fully preserved [70]. In addition, in a recent paper it was demonstrated that the biological activity of tissue type plasminogen activator released from a PEG-based hydrogel, which was obtained by ammonium persulfate and TEMED-induced polymerization of methacrylated PEG, was maintained [70]. Although the extent of oxidation can be minimized by reducing the amount of peroxydisulfate and TEMED and using methionine as antioxidant [69], other polymerization methods are certainly of interest. In recent years, UV-induced polymerization has been frequently used to prepare hydrogels [30,31,39,44,51,55,65]. Moreover, with UV-induced polymerization, gel formation can be done in situ [54], patterned structures can be prepared, and even photoreversible systems are feasible, meaning that upon exposure to UV-light the previously formed gels photodegrade whereby a drug is simultaneously released [71]. It should be noted that when the UV-polymerization is carried out in the presence of a drug, the network structure might be affected [72]. Moreover, the type of photoinitiator as well as the solvent in which it is dissolved should be selected with care, since they may leak out from the formed hydrogel. Finally, once the polymerization is carried out in the presence of a protein, the potential damage of the radicals formed on the protein structure should be assessed.

2.2. Crosslinking by chemical reaction of complementary groups

Water-soluble polymers owe their solubility properties to the presence of functional groups (mainly OH, COOH, NH_2) which can be used for the formation of hydrogels. Covalent linkages between polymer chains can be established by the reaction of functional groups with complementary reactivity, such as an amine-carboxylic acid or an isocyanate-OH/NH_2 reaction, or by Schiff base formation.

2.2.1. Crosslinking with aldehydes

Water-soluble polymers with hydroxyl groups (e.g. poly(vinyl alcohol)) can be crosslinked using glutaraldehyde (Fig. 4) [73,74]. In order to establish crosslinking, rather drastic conditions have to be applied (low pH, high temperature, methanol added as quencher). In contrast, amine containing polymers can be crosslinked with the same reagent under mild conditions whereby so-called Schiff bases are formed (Fig. 4). This has especially been investigated for the preparation of crosslinked proteins (e.g. albumin [75] and gelatin [76,77]) and amine containing polysaccharides [78]. Because glutaraldehyde is a toxic compound that even at low concentration shows cell-growth inhibition, alternatives have been developed. Crosslinking of gelatin using polyaldehydes obtained by partial oxidation of dextran has been reported [79]. These gels were designed for application in wound treatment and epidermal growth factor (EFG) was incorporated to promote wound healing. The release rate of EGF decreased with increasing storage time which was ascribed to the ongoing processes of both chemical crosslinking and physical structuring of the hydrogel matrix. Further, after prolonged storage only part of the protein was released which was likely caused by Schiff base formation between ε-lysine groups of the protein and the aldehyde groups in the oxidized dextran. The biocompatibility of dextran dialdehyde

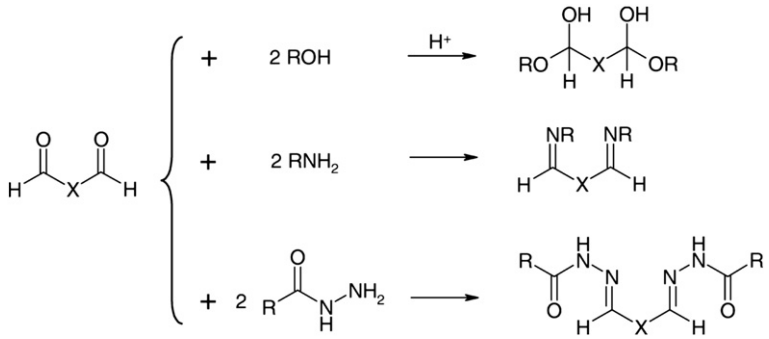

Fig. 4. Aldehyde-mediated crosslinking of polymers containing alcohol, amine or hydrazide groups (R represents the polymer chains, X is any spacer e.g. $(CH_2)_3$ in the case of glutaraldehyde).

crosslinked hydrogels was evaluated both in vitro and in vivo and was rated as acceptable [80]. Poly(aldehyde guluronate), obtained by oxidation with periodate of partially depolymerized alginate, can be converted into a hydrogel by crosslinking with adipic acid dihydrazide (Fig. 4). The swelling and degradation of the gels could be controlled by the amount of adipic acid dihydrazide [81]. When daunomycin was present during the hydrogel formation process, the drug was grafted onto the polymer matrix through a covalent linkage. Due to hydrolysis of this linkage, daunomycin was released in a time frame from 2 days to 6 weeks [82]. Hyaluronic acid hydrogel films were obtained by first derivatization of hyaluronic acid with adipic dihydrazide followed by crosslinking with a macromolecular crosslinker (poly(ethylene glycol)-propiondialdehyde). These films are enzymatically degradable with hyaluronidase and are suitable matrices for the controlled release of anti-bacterial and anti-inflammatory drugs. These hydrogels films have therefore potential to act as a delivery matrix for sustained release of drugs at wound sites [83].

2.2.2. Crosslinking by addition reactions

Water-soluble polymers can be converted into hydrogels using bis (or higher) functional crosslinking agents which react with functional groups of water-soluble polymers via addition reactions. Polysaccharides can be crosslinked with 1,6-hexamethylenediisocyanate [84], divinylsulfone [85], or 1,6-hexanedibromide [86] and many other reagents (see chapter 4 in Ref. [9]). The network properties can be easily tailored by the concentration of the dissolved polymer and the amount of crosslinking agent. The crosslinking reactions are preferably carried out in organic solvents, because water can also react with the crosslinking agent. Further, since the crosslinking agents are generally speaking very toxic, the gels have to be extracted extensively to remove traces of unreacted agents. Once these matrices are aimed for the release of pharmaceutically active agents, they have to be loaded after the gel formation and extraction process [85,86]. This means that protein molecules can be loaded in meshes of the gels which are larger than the protein and these systems therefore show typically first-order release. This often results in a limited duration of the release. Finally, between the polymer chains, linkages are established which are rather stable. This means that degradation only occurs once the polymer backbone is degraded by enzymes [20].

Recently, Hubbell and coworkers reported on a degradable hydrogel in which crosslinks were introduced by reaction of PEG-dithiol with PEG-acrylates [87]. Gel formation occurred at room temperature and physiological pH. Solid particles of a model protein (albumin) were incorporated in the gel by mixing the particles with the gel precursor solution. The gels degrade under physiological conditions due to hydrolysis of the ester bonds in the network and the degradation time (from 5 to 25 days) was controlled by the functionality of the PEG-acrylate. Albumin was released during 5–12 days.

2.2.3. Crosslinking by condensation reactions

Condensation reactions between hydroxyl groups or amines with carboxylic acids or derivatives hereof are frequently applied for the synthesis of polymers to yield polyesters and polyamides, respectively. These reactions can also be used for the preparation of hydrogels. A very efficient reagent to crosslink water-soluble polymers with amide bonds is *N,N*-(3-dimethylaminopropyl)-*N*-ethyl carbodiimide (EDC). Feijen and coworkers described the preparation of gelatin hydrogels using this reagent [88]. During the reaction *N*-hydroxysuccinimide is added to suppress possible side-reactions and to have a better control over the crosslink density of the gels. The gels were designed as a delivery device for the release of antibacterial proteins and were incorporated in a Dacron prosthetic valve. Lysozyme was loaded in the gels after their formation and released both in vitro and in vivo for a period of 2 days. To improve the loading capacity a negatively charged polysaccharide, (chrondroitin sulfate) was incorporated in the hydrogels network [89]. The loading capacity indeed substantially increased and the release is retarded with increasing chrondroitin content of the gels mostly due to electrostatic interactions between the cationic protein and anionic polysaccharide.

In order to obtain alginate gels with better mechanical properties than the ionically crosslinked gels (see Section 3.1), Mooney et al. developed a method to covalently crosslink this polymer. Alginate and PEG-diamines were crosslinked using EDC. The mechanical properties could be controlled by the amount of PEG-diamine in the gel and the molecular weight of PEG [90].

Crescezi et al. described the synthesis of polysaccharide hydrogels via the Passerini and Ugi condensation reactions (Fig. 5) [91,92]. In the Passerini condensation a carboxylic acid and an aldehyde or ketone are condensed with an isocyanide to yield an α-(acryloxy) amide. In the Ugi condensation an amine is added to this reaction mixture finally yielding an α-(acylamino) amide. The reaction can be carried out in water at slightly acidic pH and at room temperature. Since the Passerini condensation yields hydrogels with ester bonds in their crosslinks, these gels degrade at ambient temperature and pH 9.5. The degradation time varied, depending on their composition, from 1 to 8 days. Since gels prepared using the Ugi condensation contain amide bonds in their crosslinks, these gels were stable under these conditions.

Yui et al. reported on PEG-hydrogels crosslinked by a hydrolyzable polyrotaxane [93]. To obtain the gel, α-cyclodextrins were threaded on to a PEG chain capped with bulky and degradable ester end groups. Next, hydroxyl groups of the cyclodextrins were activated using carbonyldiimidazole followed by crosslinking with PEG-bisamines (Fig. 6). Due to hydrolysis of the ester groups, the gels degrade. The

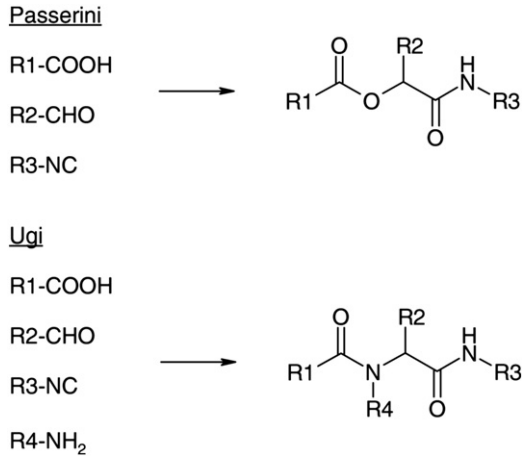

Fig. 5. Passerini and Ugi condensation reactions.

degradation time could be controlled by the gel composition and ranged from 500 to 200 h. These gels are developed to be used as scaffolds for the regeneration of soft tissue.

2.3. Crosslinking by high energy irradiation

High energy radiation, in particular gamma and electron beam, can be used to polymerize unsaturated compounds. This means that water-soluble polymers derivatized with vinyl groups (see Section 2.1) can be converted into hydrogels using high energy irradiation [29]. Hydrogels can also be obtained by radiation-induced polymerization of a mixture of a monofunctional acrylate (e.g. acryloyl-L-proline methyl ester) and a suitable crosslinker [94]. Moreover, high energy irradiation is able to crosslink water-soluble polymers without additional vinyl groups. During irradiation (gamma or electron-beam) of aqueous solutions of polymers, radicals can be formed on the polymer chain by e.g. the homolytic scission of C–H bonds. Additionally, radiolysis of water molecules generates the formation of hydroxyl radicals which can attack polymer chains also resulting in the formation of macroradicals [95]. Recombination of the macroradicals on different chains results in the formation of covalent bonds and finally in a crosslinked structure. Since the generated macroradicals can react with oxygen, radiation is normally performed in an inert (nitrogen, argon) atmosphere. Poly(vinyl alcohol) [96], poly(ethylene glycol) [97–99] and poly(acrylic acid) [100] are well known examples of polymers which can be crosslinked with high energy irradiation. The properties of the formed gels, in particular their swelling and permeability characteristics, depend on the concentration of the polymer and the radiation dose: in general, the crosslink density increases with increasing polymer concentration and radiation dose. A thermosensitive hydrogel was prepared by irradiation of aqueous solutions of poly(methyl vinyl ether) [101,102]. Irradiation of diluted polymer solutions above the phase transition temperature, yielded polymer microparticles with diameters from 300 to 500 nm [102]. Poly(amino acid)-based hydrogels were recently described and were obtained by gamma-irradiation of aqueous solutions of polypentapeptides. The polypeptides which consisted of building units of GVGVP (G=glycine, V=valine, P=proline) and related pentapeptides, were prepared by recombinant DNA technology and had molecular weights ranging from 88 to 102 kg/mol. As for other polymers, the swelling decreased with increasing radiation dose. Since poly(GVGVP) has an LCST (lower critical solution temperature) around 30 °C, the swelling was also strongly dependent on temperature [103,104].

The advantage of hydrogel formation by radiation-induced crosslinking is that this process can be done in water under mild conditions (room temperature and physiological pH). Moreover, the use of (toxic) crosslinking agents is avoided. However, the gels have to

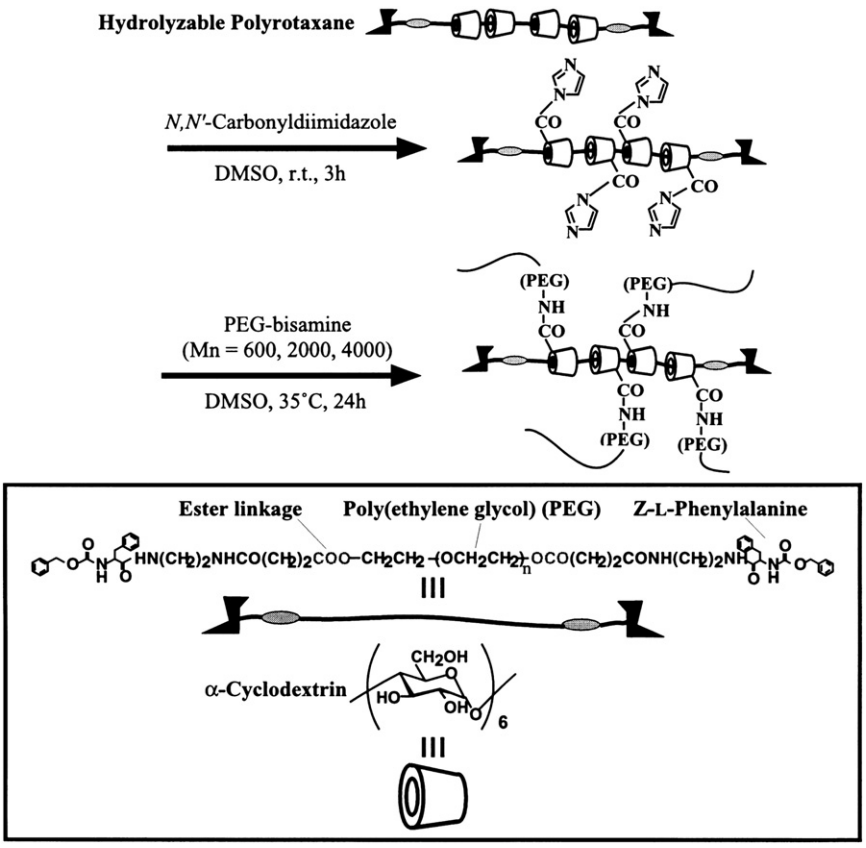

Fig. 6. Preparation of PEG hydrogels crosslinked by polyrotaxanes. Reproduced with permission from Ref. [93].

be loaded with biologically active materials after their preparation because the radicals formed during irradiation might potentially damage the biologically active substance. Further, since for example in irradiated PEG or PVA, the crosslinks consist of C – C bonds, these gels will not be biodegradable.

2.4. Crosslinking using enzymes

Sperinde et al. published an interesting method using an enzyme to synthesize PEG-based hydrogels. In their approach, a tetrahydroxy PEG was functionalized with glutaminyl groups (PEG-Q_a). PEG networks were then formed by the addition of transglutaminase to aqueous solutions of PEG-Q_a and poly(lysine-co-phenylalanine). This enzyme catalyzes the reaction between the γ-carboxamide group of the PEG-Q_a and the ε-amine group of lysine to yield an amide linkage between the polymers. The gel properties could be tailored by the ratio of the PEG-Q_a and the lysine copolymer. Under suitable conditions, gels were formed with an equilibrium water content of 90% [105]. In a recent publication, Sperinde et al. replaced poly(lysine-co-phenylalanine) by lysine end-functionalized PEG [106] and hydrogels were formed once transglutaminase was added to an aqueous solution of peptide-modified macromers. The gelation kinetics depended on the macromer structure and composition, the ratio of the reactants and the enzyme concentration. The gelation times typically were between 5 and 30 min. The gel formation occurs under very mild conditions and since the gelation kinetics can be well controlled, these systems are very suitable as in situ gelling systems.

Transglutaminases are Ca^{2+}-dependent enzymes. Based hereon, Westhaus et al. designed a triggered gelling system [107]. A mixture of Ca-loaded liposomes, fibrinogen and a Ca^{2+}-dependent transglutaminase remained fluid when stored at room temperature, but gelled rapidly when heated to 37 °C. Due to the heat-treatment the liposomes were destabilized and Ca-ions were released in the surrounding fluid simultaneously activating the enzyme. Due to the fact that this system is stable at room temperature and solidifies at 37 °C, the authors suggest that this gel system can be used as a matrix for the delivery of bioactive substances for example to promote tissue repair.

3. Physically crosslinked gels

In recent years, there has been increasing interest in physically crosslinked gels. The main reason is that the use of crosslinking agents to prepare such hydrogels is avoided. These agents can not only affect the integrity of the substances to be entrapped (e.g. proteins, cells), but these agents are often toxic compounds which have to be removed/extracted from the gels before they can be applied. To create physically crosslinked gels, different methods have been investigated.

3.1. Crosslinking by ionic interactions

Alginate is a well-known example of a polymer that can be crosslinked by ionic interactions. Alginate is a polysaccharide with mannuronic and glucuronic acid residues and can be crosslinked by calcium ions [108]. Crosslinking can be carried out at room temperature and physiological pH. Therefore, alginate gels are frequently used as matrix for the encapsulation of living cells [109] and for the release of proteins [110]. Interestingly, the gels can be destabilized by extraction of the Ca-ions from the gel by a chelating agent. The release of proteins from alginate microparticles, obtained by spraying a solution of sodium alginate into an aqueous solution of calcium chloride, can be modulated by coating the particles with cationic polymers, e.g. chitosan [111,112] and polylysine [112]. A synthetic polymer that, like alginate, can also be crosslinked with Ca-ions is poly-[di(carboxylatophenoxy)phosphazene (PCPP). Gel microbeads were prepared by spraying an aqueous solution of PCPP in an aqueous solution of calcium chloride. The ionotropic hydrogels degrade under physiological conditions. The degradation rates can be increased by incorporation of hydrolysis-sensitive glycinato groups in the polymer [113]. Polycations can be crosslinked with anions. Chitosan is a biopolymer consisting of β-(1-4)-linked glucosamine units and is obtained by deacetylation of chitin. Chitosan-based hydrogels were obtained by crosslinking of this polymer with glycerol-phosphate disodium salt [114]. Interestingly, in the presence of this salt, chitosan solutions remain liquid below room temperature, but quickly gel when heated. The temperature at which a sol–gel transition occurs decreased with increasing degree of deacetylation. Systems which are liquid at room temperature solidify at 37 °C when injected subcutaneously in rats. Biological materials, proteins and chondrocytes, were incorporated prior to the injection within the thermogelling solutions. Histological evaluations revealed that the gel can deliver active protein (BP) inducing bone and cartilage formation [114].

Crosslinking by addition of ions does not necessarily require the presence of ionic groups in the polymer for hydrogel formation. Surprisingly, dextran, which lacks ionic binding sites for cations, forms a hydrogel in the presence of potassium ions. The mechanism was elucidated by Watanabe et al. [115] who showed that the ionic radius of the potassium ion perfectly fits into the cage established by six oxygen atoms of glucose units of three polymer chains, thereby forming a microstructure. However, this dextran/potassium gel is unstable in water and therefore is less suitable for drug delivery purposes. Carrageenan, a polysaccharide composed of 1,4-linked α-D-galactose and 1,3 linked-β-D-galactose with a variable portion of sulfate groups, forms a gel with e.g. potassium ions, but also shows gelation under salt-free conditions. However, gels prepared in the presence of metallic ions were substantially stronger than those obtained under salt-free conditions [116]. In addition to anionic polymers being crosslinked with metallic ions, hydrogels can also be obtained by complexation of polyanions with polycations. Ionically crosslinked chitosan hydrogels are formed by complex formation between chitosan and polyanions, such as dextran sulfate or polyphosphoric acid [117]. Doxorubicin was encapsulated in nanoparticles of the chitosan hydrogels. These particles showed a minimal burst release and retained good in vitro cytotoxicity due to the released drug.

3.2. Crosslinking by crystallization

3.2.1. Crystallization in homopolymer systems

Poly(vinyl alcohol) (PVA) is a water-soluble polymer. When aqueous solutions of PVA are stored at room temperature they gradually form a gel with, however, a low mechanical strength. Interestingly, once aqueous solutions of this polymer undergo a freeze–thawing process a strong and highly elastic gel is formed [118]. The properties of the gel depend on the PVA molecular weight, the PVA concentration in water, the temperature and time of freezing and the number of freezing cycles. Gel formation is ascribed to the formation of PVA crystallites which act as physical crosslinking sites in the network [118]. Gels prepared using optimized conditions were stable for 6 months at 37 °C [119]. BSA loaded PVA gels were simply obtained by dissolving the protein in the aqueous PVA solution followed by freeze–thawing cycles. BSA was released by Fickian diffusion and with preservation of its conformation [120]. It was demonstrated that by the addition of alginate to the PVA solution before freeze–thawing, the gel properties could be modulated. With increasing concentration of alginate, the mechanical strength of the gel increased which was associated with a decrease in the release of a model drug [121]. In our laboratory we recently discovered that dextran 6000 spontaneously formed a hydrogel once concentrated aqueous solutions were incubated at room temperature. The gel characteristics depended on the amount of polymer in the solution. By stirring the solution, microspheres were obtained. Gel formation is most likely caused by crystallization due to association of chains through hydrogen bonding, induced in concentrated dextran 6000 solutions [122].

3.2.2. Crosslinking by stereocomplex formation

PLLA and PDLA, the homopolymers of L-lactic acid and D-lactic acid, respectively, are semicrystalline materials. High molecular weight PLA, of either stereoisomer, has a melting temperature (T_m) of 170 °C. In blends of high molecular weight PDLA and PLLA, a phase with a higher T_m (230 °C) is observed, which is ascribed to stereocomplex formation. The ability of PLA to form stereocomplexes was first described by Ikada et al. [123]. In recent years, hydrogels were described for drug delivery systems based on stereocomplex formation. In blends of triblock copolymers of PLLA–PEG–PLLA and PDLA–PEG–PDLA stereocomplex formation occurs. The release of bovine serum albumin (BSA) from microspheres based on these triblock copolymers, has been studied by Lim et al. [124] and compared with the release of BSA from microspheres prepared with one enantiomeric form of the triblock copolymer and with PLLA microspheres. The stereocomplex triblock copolymer microspheres showed a slightly larger burst release than PLLA microspheres, which is likely caused by the higher water-uptake capacity of the microspheres containing PEG. However, the release of BSA from optically pure homopolymer microspheres was not much different from that of the stereocomplex microspheres. Another system has been prepared by Lim et al. [125], based on stereocomplex formation by enantiomeric oligo(lactic acid) side chains grafted onto pHEMA (poly(HEMA-*g*-oligolactate)s). The system was prepared by casting a film from poly(HEMA-*g*-oligo(L) lactate) and poly(HEMA-*g*-oligo(D)lactate), both dissolved in chloroform. Among other characteristics, the degradation of the obtained film was compared with the degradation of a film cast from a solution of a single enantiomer of the graft copolymer. Slower degradation was observed for the 1:1 blend of the L- and D-forms than for the single enantiomer. Stereocomplex formation from water was not investigated and is most likely not possible due to both the lengths of the PLA grafts and the grafting density. In our department we designed a hydrogel system in which crosslinking is established between lactic acid oligomers of opposite chirality. It was shown that crystallinity was present in D- or L-oligomers with degree of polymerization (DP) ≥ 11. On the other hand, in blends of D- and L-oligomers of lactic acid, crystallinity (stereocomplexation) was already observed at a DP ≥ 7 [126]. In the next step, L- and D-lactic acid oligomers were coupled via their terminal hydroxyl group to dextran, yielding dex-(L)lactate and dex-(D)lactate, respectively. Upon dissolving each product in water separately and mixing the solutions, a hydrogel is formed at room temperature as was demonstrated by rheological measurements. The storage modulus of the obtained hydrogel decreased upon heating to 80 °C, while it was restored upon cooling to 20 °C demonstrating the thermo-reversibility and the physical nature of the cross-links. The storage modulus of the gels depends on the degree of polymerization of the lactate acid grafts and their degree of substitution on dextran [127]. Interestingly, gel formation was favored when one lactic oligomer was coupled via its hydroxyl group whereas the oligomer of opposite chirality was coupled via its carboxylic acid group. This is ascribed to the parallel packing of the oligomers in stereocomplexes [128]. Protein-loaded hydrogels were simply prepared by dissolving the protein in the dextran-*g*-oligolactate solutions prior to mixing. It was shown that under physiological conditions, the gels are fully degradable. The degradation time depended on the composition of the hydrogel, i.e. the number of lactate grafts, the length and polydispersity of the grafts and the initial water content, and varied from 1 to 7 days. The gels showed a release of the entrapped model proteins (IgG and lysozyme) over 6 days and the kinetics depended on the gel characteristics, such as the polydispersity of the lactate grafts and the initial water content. Importantly, the proteins were quantitatively released from the gels and with full preservation of the enzymatic activity of lysozyme, emphasizing the protein-friendly preparation method of the protein-loaded stereocomplex hydrogel [129].

3.3. Physically crosslinked hydrogels from amphiphilic block and graft copolymers

Amphiphilic block and graft copolymers are able to self-assemble in water to form organized structures like polymeric micelles and hydrogels, in which the hydrophobic segments of the polymers are aggregated. Amphiphilic diblock copolymers typically form micelles, lamellar phases, etc. [130]. Physically crosslinked hydrogels are generally obtained from multiblock copolymers or graft copolymers. The latter can be composed of a water-soluble polymer backbone, for example a polysaccharide, to which hydrophobic units are attached, or hydrophobic chains containing water-soluble grafts.

3.3.1. Block and graft copolymers of PEG and PL(G)A

The biodegradability of poly(lactic acid) (or its copolymer with glycolic acid) and the biocompatibility of poly(ethylene glycol) prompted several researchers to prepare block copolymers composed of these segments, and to develop hydrogels from them for drug delivery purposes. Drug release can be driven by both passive diffusion and degradation phenomena. The following architectures of block copolymers have been investigated: Triblock polymers with the hydrophobic PL(G)A segment in the middle have been prepared by coupling of two PEG–PL(G)A diblock copolymers [131–133]. Micelles are formed at low concentrations in water, and at higher concentrations thermoreversible gels are formed. The critical gel concentration and gel-to-sol transition temperature (also denoted as UCST) strongly depend on the molecular weights and the composition of the blocks. PEG–PLGA–PEG block copolymers also display LCST behavior, which make them interesting for drug delivery applications since the polymers form free-flowing solutions at room temperature and hydrogels at body temperature at sufficiently high concentrations (typically higher than approx. 17 wt.%) [132].

Triblock copolymers with PEG as the central block have been prepared by ring opening polymerization of lactide in the presence of PEG [134–138]. The preferred route for hydrogel formation is the phase separation method, i.e. addition of water to a solution of the polymer in tetraglycol [139]. The water-miscible solvent was removed by washing with water. It was suggested that the hydrogels can accommodate both hydrophilic drugs in the swollen PEG phase as well as hydrophobic drugs in the PLA domains. Furthermore it was observed that the hydrogels are soft enough to be injectable. Bovine serum albumin (BSA) and fibrinogen showed different release behaviors. In contrast to BSA, fibrinogen displayed almost linear release profiles, indicating a reservoir drug delivery system, probably due to incompatibility of the protein with the aqueous PEG phase. Microspheres of triblock and star-branched block copolymers have been prepared by Kissel et al. and their swelling and degradation behavior [140,141] as well as the use as protein delivery system [142–144] have been extensively studied. The microspheres showed continuous mass loss and decrease in molecular weight of the polymers. As a consequence, after a small burst release a continuous release of BSA was observed, which depended on the polymer composition, pH, and ionic strength [143].

Multiblock copolymers of PEG and PLGA were prepared by polycondensation of dicarboxylated PLA and PEG [145,146]. Block copolymers with relatively small PLA blocks are soluble in water and exhibit LCST behavior, the phase transition temperature depending on the PLA molecular weight. The polymers which are not soluble do however swell in water. Preliminary results showed improved wound healing when wounds of rats were covered with dried films of the multiblock copolymer containing basic fibroblast growth factor (bFGF), indicating at least partial preservation of bFGF bioactivity [146]. The properties of multiblock copolymers of PEG and PLA were compared with those of PEG and poly(ε-caprolactone) (PCL). For the PCL containing materials, reversible swelling/deswelling was observed with alternating low and high temperature, respectively,

which was attributed to enhanced hydrophobic interactions of the PCL blocks at high temperature (LCST behavior). The swollen but not heat-exposed PEG–PCL hydrogels showed much higher strengths than the PEG–PLA hydrogels due to the high crystallinity of the PCL segments.

Graft copolymers of PEG and PLA have been described both with a PLGA backbone and PEG grafts as well as the other way around [147]. A hydrogel from the former was reported to give only partial degradation and protein release, while the polymers with a PEG backbone and PLGA grafts resulted in hydrogels which totally disintegrated after 7 days. The phase diagram of the latter was very similar to those of PEG–PLGA–PEG triblock polymers mentioned above, i.e. displaying a sol-to-gel transition temperature above a critical gel concentration of 16 wt%.

3.3.2. Block copolymers of PEG and PBT

Multiblock copolymers of PEG and another hydrophobic polyester, poly(butylene terephthalate) (PBT), were investigated by Feijen and coworkers [148–152]. These biocompatible polymers are prepared by melt polycondensation of PEG, butanediol and dimethyl terephthalate. To load polymers with lysozyme as a model protein, polymer solutions were prepared in a mixture of chloroform and hexafluoroisopropanol (6:1) and subsequently a water-in-oil emulsion was prepared containing the protein in the aqueous phase. These emulsions were either cast to form a film, or microspheres were prepared using a water-in-oil-in-water emulsion method. The equilibrium swelling ratio, the estimated mesh size of the hydrogel network and the release rate of lysozyme increased with the weight percentage and molecular weight of the PEG blocks [148,149]. The hydrolytic degradation of the polymers was studied. A polymer with PEG block molecular weight of 1000 g/mol and 30 wt.% of PBT showed about 25% weight loss after a period of 35 days. With increasing PBT wt.% the degradation rate decreased. Lysozyme was almost completely released from the former polymer in about 1 week, with no loss of its enzymatic activity during preparation, storage and release. The diffusion coefficients strongly depended on the composition of the copolymers. Control of protein release was possible by adjusting the water/polymer (w/p) ratios during the emulsification in the preparation process of the films and microspheres [150]. First-order release of lysozyme was observed for copolymers containing PEG 4000, whereas near zero-order release was observed for polymers with smaller PEG blocks when w/p ratios were below 1 ml/g during emulsification. The latter was similar to the release profile of fibrinogen from the previously mentioned PLA–PEG–PLA hydrogels, however a different explanation was given by the authors, i.e. an enhanced release rate in time due to degradation of the hydrogel compensating for the reduced drug concentration.

3.3.3. Hydrophobized polysaccharides

Examples of polysaccharides reported in literature used for the preparation of physically crosslinked hydrogels by hydrophobic modification are chitosan, dextran, pullulan and carboxymethyl curdlan. The group of Sunamoto and coworkers has concentrated on hydrogels based on cholesterol-bearing pullulan [153–159]. This compound forms monodisperse hydrogel nanoparticles of 20–30 nm with high water content (typically about 80 wt%) upon dialyzing a solution from DMSO against PBS buffer. Various proteins such as α-chymotrypsin, BSA and insulin have been incorporated. As an example, the hydrophobic antitumor drug adriamicin (ADR) was taken up inside the particles by simply mixing the pullulan suspension with ADR [155]. Slow release was observed at pH 7.4, which increased at lower pH of the medium due to increased solubility of the drug. The stability of the drug was increased, however due to poor cellular uptake of the complexes and/or slow release of ADR, the in vitro cytotoxicity against HeLa cells appeared rather low. Rapid complexation was also observed for insulin: approximately 10 molecules of insulin were taken up inside the pullulan particles within 10 min after mixing [156]. The resulting microenvironment protected the protein effectively against thermal denaturation, aggregation and enzymatic degradation. Dissociation of insulin occurred on addition of BSA, which may provide a basis for in vivo release of the insulin. Indeed, the in vivo activity of the insulin-loaded nanoparticles was similar to that of free insulin after i.v. injection. By covalent attachment of lactoside or galactoside to the pullulan backbone, nanoparticles were obtained which show binding to the receptor β-D-galactose-specific lectin RCA_{120}, which provides a possibility for cell-specific targeting [158]. In vitro they were more effectively internalized than conventional nanoparticles by cells expressing galactose-specific receptors such as rat hepatocytes and HepG2 cells, in contrast to cells which lack such receptors [158]. Moreover, biodistribution studies showed enhanced accumulation in the liver.

Self-assembled hydrogel nanoparticles have also been prepared from carboxymethyl (CM) curdlan, a polysaccharide having anti-tumor activity, by substitution with a hydrophobic sulfonylurea [160]. Release of the anti-cancer drug all-*trans* retinoic acid (ATRA) from these nanoparticles showed first-order-like kinetics. An increased drug-loading and degree of hydrophobic modification resulted in a slower release. Upon conjugation of lactobionic acid to the CM-curdlan, specific binding to HepG2 cells was observed in vitro.

Another example of a hydrophobized polysaccharide is the water-soluble glycol chitosan substituted with palmitoyl chains. They assemble into unilamellar polymeric vesicles in the presence of cholesterol [161]. These polymeric vesicles are found to be biocompatible and haemocompatible and capable of entrapping water-soluble drugs [162]. Highly porous solid materials are obtained after freeze drying, which are hydrated without swelling to 20× its dry weight in alkaline buffer [163]. In contrast to most chitosan-based materials, the hydration decreased in acidic environment, which was attributed to the higher erosion rate at low pH.

Chitosan has been grafted with PL(G)A by Qu et al., in which the hydrophobic polyester side chains are responsible for the hydrophobic interactions in water [164,165]. Reversible water uptake of the materials was observed when the pH was switched between 2.2 and 7.4, the lowest pH providing the highest swelling due to charge repulsion by protonation of the free amine groups on the polymer backbone. The maximum water uptake in the protonated form depends on the glycolic acid/lactic acid content. Other chitosan hydrogels that respond to external changes like pH or temperature have been prepared by grafting with poly(acrylic acid) (PAAc) [166] or poly(*N*-isopropylacrylamide) (PNIPAAm) [167]. In addition to chitosan, also carboxymethyl dextran has been grafted with PNIPAAm chains aiming at thermosensitive hydrogels [168]. Cloud point measurements were conducted in this case, indicating a phase transition at around 38 °C, but no data on hydrogel characteristics have been reported yet.

3.3.4. Other block and graft copolymers

Recent novel examples of physically crosslinked hydrogels from block copolymers are: (multi)block copolymers of PEG-polyisobutylene [169], PEG-poly(γ-benzyl L-glutamate) [170], poly(2-ethyl-2-oxazoline)-PCL which behaved like PEG–PCL hydrogels (vide supra) [171], and thermosensitive hydrogels from PEG–PNIPAAm [172]. The latter consist of triblock and star copolymers derived from linear and multi-arm PEG as the water-soluble central block and PNIPAAm as the thermosensitive terminal blocks. Injectable low-viscous solutions were obtained in water at room temperature, which form a thermoreversible network gel at body temperature in less than 1 min upon passing the cloud point temperature of PNIPAAm.

Superabsorbing hydrogels based on hydrophobic crosslinking were obtained from poly(*N*-vinylpyrrolidinone-*g*-styrene) [173]. Materials were obtained which swell up to about 33× their dry mass resulting in hydrogels containing 97% water. Finally, the irreversible physical crosslinking of PMMA microemulsion particles as reported by Ming et al. can also be considered as due to hydrophobic interactions

[174]. The self-assembly of the nanoparticles into a network structure was suggested to be due to the insufficient surface coverage of the surfactants used during the microemulsion polymerization process. As much as 86.4 wt% of water can be immobilized inside the network.

3.4. Crosslinking by hydrogen bonds

Poly(acrylic acid) and poly(methacrylic acid) form complexes with poly(ethylene glycol). These complexes are held together by hydrogen bonds between the oxygen of the poly(ethylene glycol) and the carboxylic group of poly((meth)acrylic acid), whereas for poly((meth)acrylic acid) hydrophobic interactions also play a role [175]. Hydrogen bonding does not only occur between poly((meth) acrylic acid) and poly(ethylene glycol), but has also been observed in poly(methacrylic acid-*g*-ethylene glycol) [176,177]. The hydrogen bonds are only formed when the carboxylic acid groups are protonated. This implies that the swelling of these gels is strongly dependent on the pH. Moreover, the complex of poly(methacrylic acid) and poly(ethylene glycol) prepared at low pH can be dissolved in ethanol. Upon injection, the diffusion of ethanol from the liquid transforms the system into a gel. The gel gradually dissolves in time due to dissociation of the complex [178].

The double strands in DNA are held together by hydrogen bonding and stacking of bases. Nagahara et al. made use of this principle by developing a hydrogel system in which crosslinking was established by hybridization [179]. In their approach, oligodeoxyribonucleotides were coupled to a water-soluble polymer (poly(*N*,*N*-dimethylacrylamide-co-*N*-acryolyloxysuccinimide). Hydrogels were formed by the addition of a complementary ODN either conjugated to the same water-soluble polymer or in its free form, to an aqueous solution of the ODN-derivatized water-soluble copolymer. The gels were formed at room temperature, but dissociated at higher temperatures.

3.5. Crosslinking by protein interactions

3.5.1. Use of genetically engineered proteins

Protein engineering is a new development in materials chemistry, a field which was pioneered by Tirrell and Cappello [180,181]. The

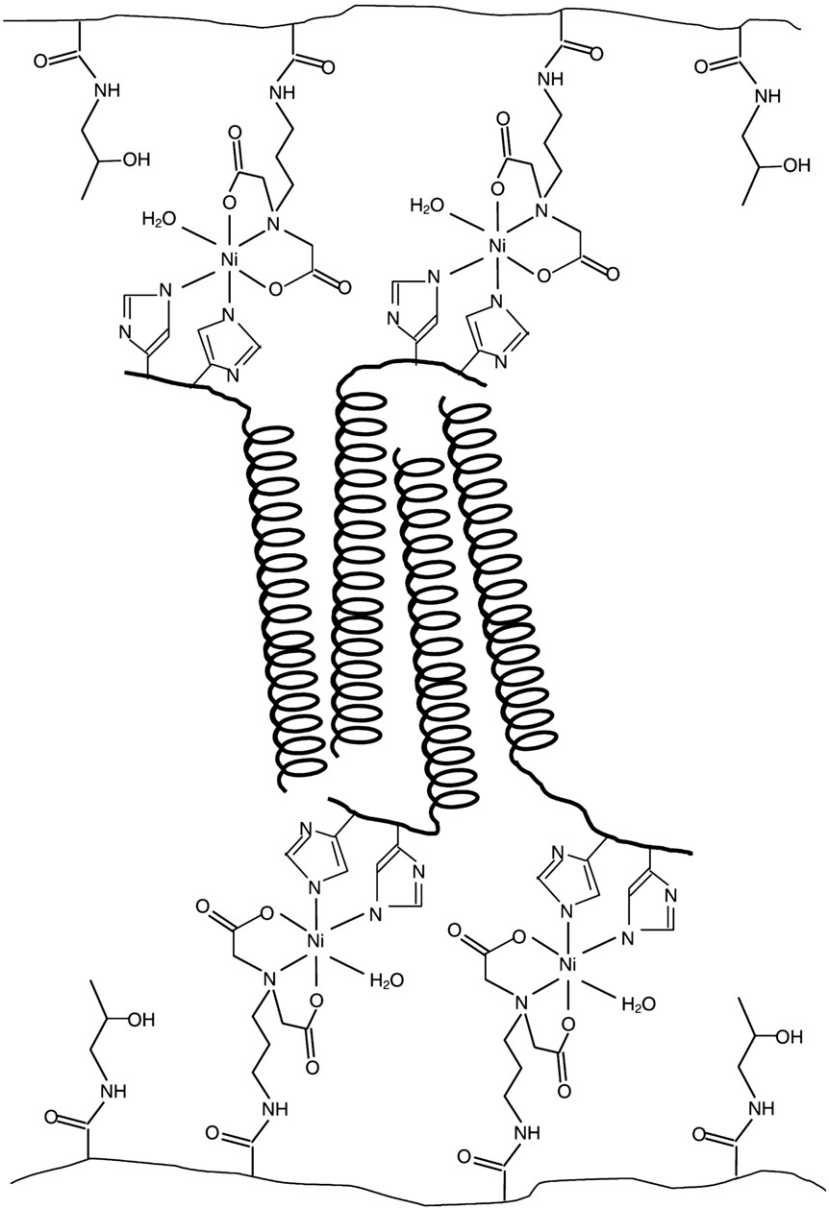

Fig. 7. Structural representation of the hybrid hydrogel primary chains and the attachment of His-tagged coiled-coil proteins. Reproduced with permission from Ref. [185].

major advantage is that the sequence of peptides and thereby its physical and chemical properties can be precisely controlled by the proper design of the genetic code in synthetic DNA sequences. Even synthetic amino acids which normally do not occur in nature can be employed [182].

By genetic engineering, Cappello and colleagues prepared sequential block copolymers containing a repetition of silk-like and elastine-like blocks, in which the insoluble silk-like segments are associated in the form of aligned hydrogen bonded beta strands or sheets [181,183]. These biocompatible so-called ProLastins are fluid solutions in water which can be mixed with drugs, and undergo an irreversible sol-to-gel transition with time under physiological conditions due to crystallization of the silk-like domains. The rate of gelation and subsequent drug release depends on various factors like concentration, polymer composition, and temperature. The release rates are related to water content of the gels and the molecular weights of the incorporated compounds and follow first-order like kinetics.

Some natural proteins form so-called coiled coils, i.e. left-handed superhelices of two or more right-handed α-helices. These α-helices are characterized by a hydrophilic and a hydrophobic side due to a typical repeating sequence of amino acids with different polarities. The proteins undergo conformational transition in response to, for example, temperature and pH. A polypeptide showing this behavior has a so-called 'leucine zipper' motif and was connected to a water-soluble central polypeptide block as part of a triblock architecture, designed and genetically engineered by Tirell and coworkers [184]. The hydrogel formed by the coiled-coil interactions gradually turned into a viscous solution with increasing temperature and when pH was increased above 8.0.

Kopeček and coworkers investigated natural and engineered proteins which show coiled-coil interactions and used them as crosslinkers for poly(*N*-(2-hydroxypropyl)methacrylamide) (PHPMA) [185,186]. The proteins were attached with one end to the polymer backbone by metal complexes between histidine tags and metal-chelating ligands on the polymer (Fig. 7). The hydrogel including the natural protein showed a temperature-induced collapse close to the melting temperature of the coiled-coil protein (35 °C), which was attributed to the change from an elongated rodlike coiled-coil conformation to random coils. The engineered protein and the hydrogel composed from it did not show such a phase transition.

The unfolding of a beta-sheet structure above the melting temperature of the I28 Ig-like module of a human cardiac muscle protein was used as a reversible crosslinking method [187]. PHPMA hydrogels were prepared in a similar fashion as described above, and as a result of the melting of the crosslinks they swelled to three times their initial volume at elevated temperatures.

3.5.2. Crosslinking by antigen–antibody interactions

Miyata et al. prepared a hydrogel in which an antigen (rabbit IgG) was grafted to chemically crosslinked polyacrylamide in the presence of antibody as an additional crosslinker [188]. The hydrogel lightly swelled in the presence of free antigen due to the replacement of polymer-bound antigen, resulting in the release of the antibodies and thereby decrease in crosslink density. The swelling ratio increased with increasing antigen concentration, but the process was not fully reversible due to the loss of the antibodies from the hydrogel. Therefore, another hydrogel was prepared by immobilizing both the antigen and the antibody in the form of an interpenetrating polymer network [189]. Indeed, reversible swelling/deswelling was observed upon alternating exposure of the hydrogel to antigen-containing and antigen-free solutions. Furthermore, hydrogel membranes were prepared displaying on/off switching behavior with respect to protein (haemoglobin) permeation through the membranes, suggesting that this approach might permit drug delivery in response to a specific antigen.

4. Conclusions

In recent years, many novel hydrogel systems have been developed. Fundamental studies greatly contributed to our present understanding of this unique class of materials. Also in terms of application, great progress has been made and hydrogels are under investigation, among others, as matrices for the encapsulation of living cells and for the controlled release of pharmaceutically active proteins. Many crosslinking methods have been developed and are presently available for the preparation of hydrogels. For the entrapment and encapsulation of labile bioactive substances and living cells, physically crosslinked gels are of great interest, especially once the gel formation occurs under mild conditions in the absence of organic solvents. Although a number of interesting physical methods is presently known, there is certainly a need for other methods. It is expected that principles from the expanding research area of supra-molecular chemistry will be applied to design novel type of hydrogels with tailored properties which can preferably be prepared in an aqueous environment. Also, protein engineering might contribute to the development of hydrogel systems with a very precise control over their micro structure and thus their properties. Finally, it can be foreseen that systems in which gel formation is induced by a specific trigger (temperature, pH or a specific compound) will be further developed and applied for pharmaceutical and biomedical purposes.

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