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Review

Hydrogels in a historical perspective: From simple networks to smart materials

Sytze J. Buwalda [a,*,1], Kristel W.M. Boere [a,1], Pieter J. Dijkstra [b], Jan Feijen [c,d,e], Tina Vermonden [a], Wim E. Hennink [a]

[a] Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands
[b] Department of Developmental BioEngineering, Faculty of Science and Technology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands
[c] Department of Polymer Chemistry and Biomaterials, Faculty of Science and Technology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands
[d] Biomedical Polymers Laboratory, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China
[e] Jiangsu Key Laboratory of Advanced Functional Polymer Design and Application, Department of Polymer Science and Engineering, College of Chemistry, Chemical Engineering and Materials Science, Soochow University, Suzhou 215123, PR China

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ABSTRACT

Over the past decades, significant progress has been made in the field of hydrogels as functional biomaterials. Biomedical application of hydrogels was initially hindered by the toxicity of crosslinking agents and limitations of hydrogel formation under physiological conditions. Emerging knowledge in polymer chemistry and increased understanding of biological processes resulted in the design of versatile materials and minimally invasive therapies. Hydrogel matrices comprise a wide range of natural and synthetic polymers held together by a variety of physical or chemical crosslinks. With their capacity to embed pharmaceutical agents in their hydrophilic crosslinked network, hydrogels form promising materials for controlled drug release and tissue engineering. Despite all their beneficial properties, there are still several challenges to overcome for clinical translation. In this review, we provide a historical overview of the developments in hydrogel research from simple networks to smart materials.

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* Corresponding author at: Laboratoire Hétérochimie Fondamentale et Appliquée, Université Paul Sabatier/CNRS UMR 5069, 118 Route de Narbonne, 31062 Toulouse Cedex 09, France.
[1] Both authors contributed equally.

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1. Introduction

Hydrogels are three-dimensional polymer networks that are able to retain a large amount of water in their swollen state [1]. Hydrogels may be classified as natural, synthetic or hybrid, depending on the source of the constituting polymers. Hydrogels can be chemically crosslinked by covalent bonds, physically crosslinked by non-covalent interactions or crosslinked by a combination of both. The interactions responsible for the water sorption include capillary, osmotic and hydration forces, which are counterbalanced by the forces exerted by the crosslinked polymer chains in resisting expansion [2]. The equilibrium swollen state depends on the magnitudes of these opposing effects, and determines to a large extent some important properties of the hydrogel, including internal transport and diffusion characteristics, and mechanical strength. Many of these properties are governed not only by the degree of swelling, but also directly by the chemical nature of the polymer network and the network morphology. Due to their high water content, the properties of hydrogels resemble those of biological tissues, resulting in an excellent biocompatibility. Furthermore, their soft and rubbery nature minimizes inflammatory reactions of the surrounding cells [3]. After their discovery in the 1960s by Wichterle and Lim [4] hydrogels were first successfully applied as contact lenses. Later, hydrogels have been frequently used as systems for the controlled delivery of biologically active agents. These hydrogels facilitate the localized and sustained release of a drug, thereby decreasing the number of administrations, preventing damage to the drug and allowing for relatively low doses. In this field, the Journal of Controlled Release has played a major role since its launch in 1984 as a place to publish state-of-the-art research and review articles concerning drug delivery from hydrogels. In this contribution for the 30th Anniversary Issue of the Journal of Controlled Release, we present a historical overview of the major developments in hydrogel research over the last 50 years, starting with the relatively simple, chemically crosslinked networks of the 1960s and concluding with today's 'smart' hydrogels (Fig. 1). We particularly focus on hydrogels for controlled drug delivery, but we also briefly address hydrogels for other biomedical applications such as tissue engineering. Lastly, we present our view on the future of hydrogel research.

Because of the vastness of this research field, obviously not all contributions of the last 50 years could be included in this historical overview. However, many excellent reviews exist that focus on specific areas in hydrogel research [5–14].

2. First generation hydrogels

Around 1900, the term 'hydrogel' first appeared in scientific literature when it was used to describe a colloidal gel of inorganic salts [15]. In 1960, Wichterle and Lim were the first to report on hydrogels as we know them nowadays, e.g. as water-swollen crosslinked macromolecular networks, in their landmark paper about poly(2-hydroxyethyl methacrylate) (pHEMA) gels for use as soft contact lenses [4]. In the two decades following this discovery, hydrogel research remained essentially focused on relatively simple, chemically crosslinked networks of synthetic polymers with applications mainly in ophthalmic and drug delivery research. The straightforward network structure was also well-suited for fundamental characterization and modeling of various physico-chemical hydrogel properties such as solute diffusivity and crosslink density. Hydrogels were mainly prepared either by polymerization of water-soluble monomers in the presence of a multifunctional crosslinker or by crosslinking of hydrophilic polymers (Fig. 2). These categories among the first generation of hydrogels will be discussed separately hereafter including the most representative examples. The chemical structures of the polymers that were applied most frequently in hydrogels, pHEMA, poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG), are shown in Fig. 3.

2.1. Hydrogels prepared by polymerization of water-soluble monomers

The hydrogels in this category are prepared by chain-addition reactions, mostly employing vinyl monomers. The mechanism of this type of polymerization has been well established [16] and, in short, consists of an initiating free radical species which adds to a vinyl monomer molecule by opening the π-bond to form a new radical, until the polymerization is terminated at some point by recombination of two radical species or disproportionation. The polymerization of a monomer in the presence of a crosslinking agent in solution has various advantageous aspects over bulk polymerization, such as rapid hydrogel formation under mild conditions and the possibility to obtain pre-defined shapes because the starting materials are in the liquid form [17].

An important hydrogel-forming polymer in terms of production volume in this category is poly(acrylamide) (PAM), which was initially employed mainly in industrial applications such as agricultural gels. In the 1960s, PAM hydrogels were also used for the physical entrapment

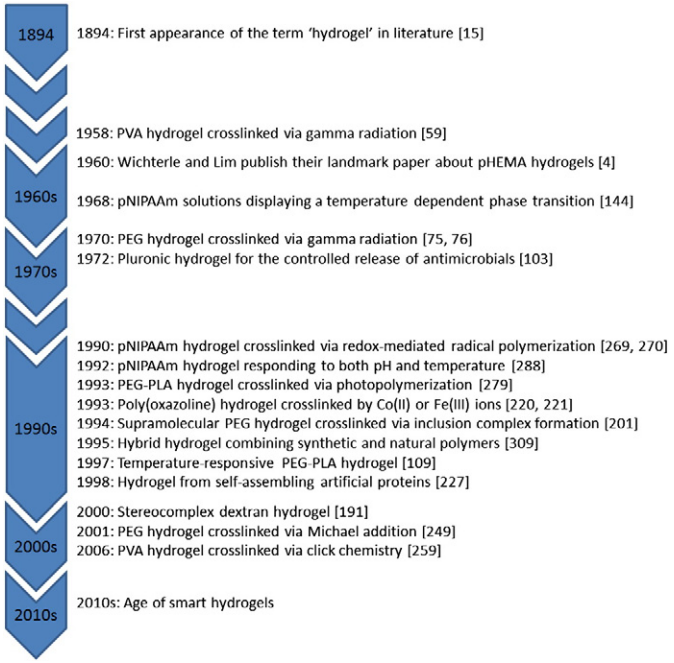

Fig. 1. Timeline presenting the most important events in the history of hydrogel research.

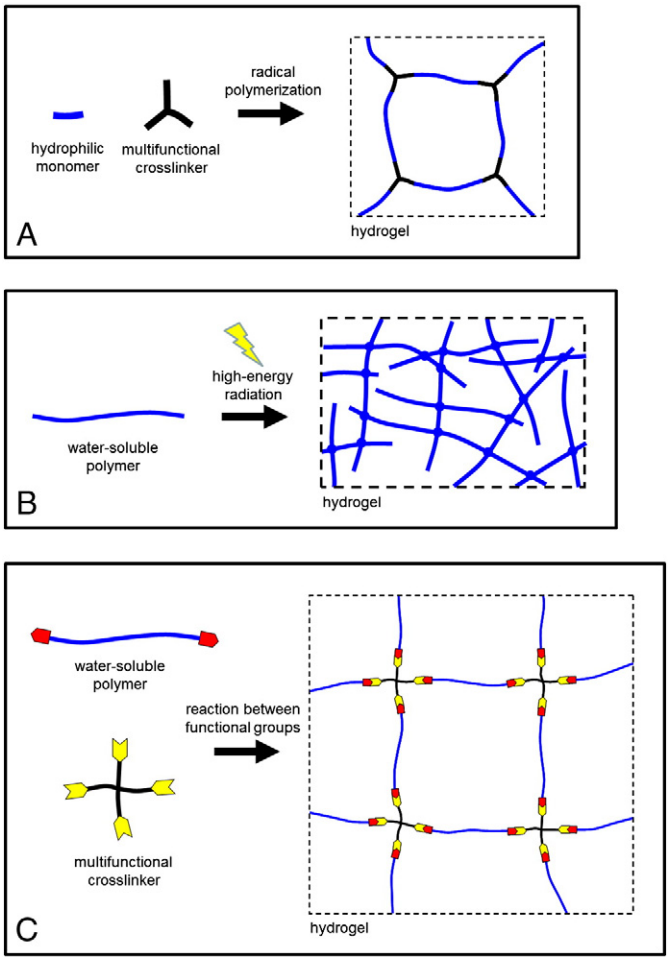

Fig. 2. Main strategies for the preparation of the first generation hydrogels. (A) Polymerization of water-soluble monomers in the presence of a multifunctional crosslinker. (B) Crosslinking of hydrophilic polymers by radiation. (C) Crosslinking of hydrophilic polymers by reaction between complementary groups.

of cells [18] and enzymes [19], as well as for the covalent attachment of proteins; only recently PAM gels have found widespread biomedical application as soft tissue fillers and augmentation materials [20]. The most extensively studied polymers for application in the first generation biomedical hydrogels were the poly(hydroxyalkyl methacrylate)s.

2.1.1. Poly(hydroxyalkyl methacrylate)s

Between 1950 and 1955, Wichterle postulated the fundamental conditions required for synthetic materials in direct contact with living tissues [21]. These included: the absence of extractable impurities; mechanical properties similar to those of the surrounding tissue; sufficient permeability for water-soluble substances such as salts, proteins and oxygen; and resistance to degradation by enzymatic systems. Driven by these requirements, Wichterle and Lim prepared their first hydrogel in 1960 by free radical polymerization of 2-hydroxyethyl methacrylate (HEMA) in aqueous solution with ethylene glycol dimethacrylate (EGDMA) as a crosslinker [4]. These hydrogels found widespread use as soft contact lenses throughout the 1960s, but disadvantages such as insufficient oxygen transport and mechanical fragility sparked research toward improved pHEMA hydrogels [22]. Most notably, from 1970 onwards N-vinylpyrrolidone (NVP) was used to increase the biocompatibility because of its high hydrophilicity, exceeding that of HEMA [23]. NVP was employed as a comonomer together with HEMA [24] or as polymerized PVP grafted onto pHEMA [25]. Both approaches resulted in improved oxygen permeability and wettability of contact lenses, but reports on PVP loss over time [26] led to a preference for copolymerization of HEMA with NVP. Although p(HEMA-co-NVP) contact lenses exhibited several improved properties over pHEMA lenses, the poor copolymerization of methacrylate monomers with NVP due to a large difference in reactivity ratio resulted in hydrogels with an often inconsistent quality in terms of dimensional control, modulus and water content [27]. Therefore, many alternative comonomers were investigated since the late 1970s for copolymerization with HEMA, including substituted acrylamides [28], vinyl acetate [29] and substituted methacrylates [30]. Since then significant improvements have been achieved and nowadays materials can be created with various water contents, refractive indices, mechanical properties and oxygen permeabilities. Fifty years after its discovery, pHEMA still remains the basis for many contact lenses [31,32].

pHEMA hydrogels were also applied in controlled drug delivery applications, as a result of their hydrolytic stability and the possibility to modulate the release properties by introducing co-monomers and

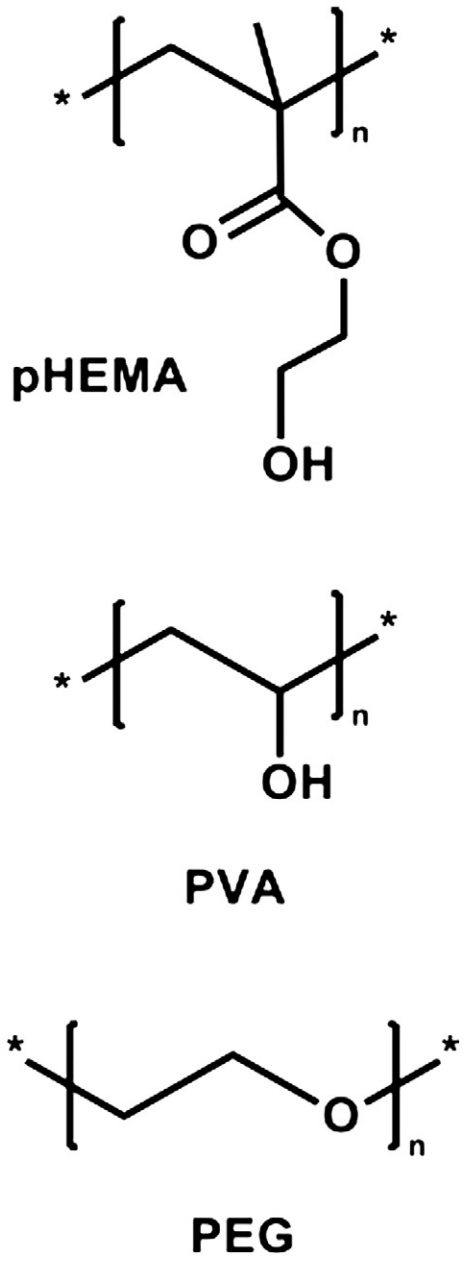

Fig. 3. Chemical structures of the polymers that were applied most frequently in hydrogels of the first generation.

varying the crosslink density [33]. Early pHEMA hydrogels possessed a homogeneous network structure and water distribution resulting in optical clarity, which made them suitable for application as contact lenses [34], as discussed above. The polymerization method in aqueous solution, however, gives rise to a relatively small mesh size, on the scale of nanometers, resulting in limited transport of high-molecular weight compounds [35]. To increase the effective mesh size, in 1972 a freeze–thaw technique was described in which HEMA was polymerized around ice crystals that were subsequently thawed, resulting in macroporous hydrophilic pHEMA membranes [36]. This technique was later expanded for the preparation of pHEMA hydrogels with pore sizes of several micrometers enabling the controlled delivery of macromolecular drugs [37]. Since the 1970s pHEMA based hydrogels have been employed for the controlled release of agents from various drug classes, including anti-arrythmics [38], contraceptives [39], antibiotics [40], ophtalmics [41], vasoconstrictors [42], anti-inflammatory drugs [43] and cytostatics [44]. Hydrogels based on pHEMA were also examined in the late 1960s as blood compatible materials [45,46] and as bulk space fillers in reconstructive surgery [47,48].

Several physical properties of pHEMA hydrogels relevant for the above-mentioned biomedical applications, such as swelling behavior and crosslink density, were studied in detail during this period, most prominently by the group of Peppas [49]. The diffusional characteristics in pHEMA hydrogels of various substances such as oxygen [50], water [51] and model solutes like NaCl, urea, glucose and lysozyme [52,53] were also investigated.

2.2. Hydrogels based on crosslinking of water-soluble synthetic polymers

The second category of the first generation hydrogels is constituted by hydrophilic polymers that are covalently crosslinked either by reaction between functional groups or by free radicals. Two polymers have been studied extensively in this category, namely poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG).

2.2.1. PVA

PVA, first described in 1924, is a linear synthetic polymer produced by free radical polymerization of vinyl acetate to poly(vinyl acetate) and subsequent hydrolysis of the acetate groups to alcohol groups [54]. The extent of hydrolysis, usually between 85 and 100%, determines various PVA properties such as crystallinity and aqueous solubility, which are important parameters determining the hydrogel properties. PVA hydrogels have mostly been prepared by chemical crosslinking. Various bi- or multifunctional crosslinkers with groups reactive to the hydroxyl groups of PVA were used for the synthesis of chemical gels, including aldehydes [55], anhydrides [56] and isocyanates [57]. The models developed by Peppas for determination of the molecular weight between crosslinks, M_c, employing swelling experiments or dynamical mechanical analysis, were applied to PVA hydrogels crosslinked by glutaraldehyde. M_c values typically ranged between 400 and 8000 g/mol depending on the relative amount of crosslinker (Fig. 4) and the type of experiments performed [55]. The main concern with this crosslinking method is the possible presence of residual crosslinking agents causing adverse effects for biomedical applications [58]. It was demonstrated in the 1960s that irradiation of aqueous PVA solutions by gamma or electron beam radiation also results in the formation of covalently crosslinked hydrogels [59,60]. The formation of free carbon radicals along the backbone and subsequent network formation by recombination of polymer radicals was proposed as the mechanism behind radiation-mediated crosslinking. This crosslink method was considered cleaner and safer than methods involving bifunctional crosslinking agents as mentioned above [61], with the additional advantage of simultaneous sterilization during hydrogel formation. However, early examples of radiation-crosslinked PVA hydrogels suffered from poor mechanical properties [62]. Reinforcement was achieved by an annealing process, which introduces crystallites in the polymeric network, but as a result the optical clarity of the hydrogel was lost [63]. Physical PVA hydrogels, prepared by repeated freeze–thaw cycles to densify the macromolecular structure, have been reported since the 1970s [64,65], but the crystallization mechanism behind PVA cryogel formation, as well as their eventual applications such as in tissue engineering, were recognized only in the 1990s [66].

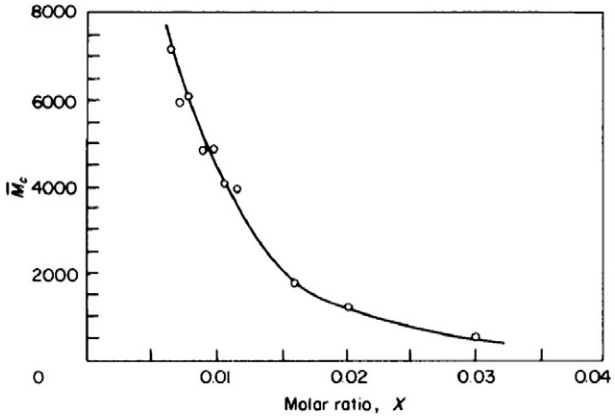

Fig. 4. Molecular weight between crosslinks M_c versus the molar ratio X glutaraldehyde/PVA repeating unit.
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PVA hydrogels were widely studied for use in cardiovascular surgery or other blood-handling procedures, most notably by the group of Sefton. PVA hydrogels were frequently combined with heparin, a natural sulphated glycosaminoglycan, which displays anticoagulant activity by inactivating blood coagulation factors such as thrombin [67].

PVA based systems were also studied as controlled drug delivery devices, mainly as swelling-controlled hydrogels, which are initially in the dry or glassy state and start to release the drug upon swelling once exposed to biological fluids. PVA hydrogels crosslinked with glutaraldehyde released the model drug theophylline with near zero-order kinetics at a rate that could be controlled by the crosslink density [68]. (Compressed) blends of PVA with other homopolymers such as PVP and PEG were shown to release model drugs of varying sizes in a controlled manner, and zero-order release was achieved for some formulations [69,70]. The influence of a number of parameters, such as crosslink density and hydrogel morphology, on the diffusion of various model solutes through PVA hydrogels was discussed both theoretically and experimentally in several papers [68,71,72].

2.2.2. PEG

PEG received considerable attention for use in hydrogels during the 1960s and 1970s because of its good biocompatibility and resistance to protein adsorption [73]. Although the terms poly(ethylene glycol) (PEG) and poly(ethylene oxide) (PEO) have been used inconsistently in hydrogel literature, the term PEG refers to polymer chains with lower molecular weights where the hydroxyl end groups still contribute significantly to a glycolic chemical nature, while PEO refers to higher molecular weight polymers where the polyether character dominates [74]. The cutoff is debated but is generally set at 20 kg/mol [74]. For convenience, the term PEG will be used throughout this section. In the early years of hydrogel research, approaches for the preparation of hydrogels from the highly water-soluble PEG homopolymer mostly concerned covalent crosslinking methods. It was shown that aqueous solutions of PEG can be crosslinked under gamma or electron beam radiation to form a hydrogel [75,76]. The irradiation technique was used by a number of research groups to prepare PEG hydrogels, from both linear and star-shaped PEG [77–79]. Hydrogel formation was also achieved by reaction of PEG end groups with end groups on other PEG macromonomers or low molecular weight crosslinkers.

Early examples of specific chemical reactions include free radical polymerization of methacrylate groups [80,81] and reaction between isocyanates and alcohols [82]. More recently, condensation reactions [83], Michael-type addition [84], click chemistry [85], native chemical ligation [86] and enzymatic reactions [87] have also been employed, which will be addressed in a subsequent paragraph of this review (Section 5.1). Physical crosslinking techniques for PEG homopolymers were described as well, such as the formation of association complexes of PEG with poly(methacrylic acid) [88], but no applications were reported. Hydrogels based on block and graft copolymers of PEG, such as poly(ethylene glycol)–b-poly(propylene glycol)–b-poly(ethylene glycol) (Pluronics or Poloxamers) [89], started to emerge during the 1960s, but these materials form a class of its own and will be reviewed in Section 3.1.1.

Early PEG hydrogels were applied mostly as controlled drug delivery systems and as anti-adhesive biomaterials. Graham et al. designed hydrogels by crosslinking PEG diisocyanates with hexanetriol for the sustained release of prostaglandin E2 [90], caffeine [91] and morphine [82]. A diffusion controlled release was obtained, which could be tuned by the amount of crosslinker, the PEG M_n and the device geometry (Fig. 5). Promising clinical data were reported for prostaglandin releasing PEG hydrogels regarding the induction and facilitation of child delivery for patients with an unripe cervix [80]. A contraceptive sponge with the brand name Today, based on crosslinked PEG diisocyanates and containing the spermicide nonoxynolphenol, was commercialized in the beginning of the 1980s and is still on the market today [92]. The same holds for Vigilon, a radiation crosslinked PEG hydrogel supported by a PE net which is sold as a sheet wound covering material, exploiting the biocompatibility and inertness of PEG [93]. These properties also prompted several investigators to study PEG hydrogels as tissue engineering matrices. Examples include photopolymerized hydrogels based on PEG methacrylates for the encapsulation of yeast cells [80] and enzymes [81].

2.3. Hydrogels based on cellulose

Besides entirely synthetic polymers such as pHEMA, PEG and PVA, semi-synthetic derivatives of the natural polymer cellulose also received attention for use in biomedical applications, predominantly for the controlled delivery of drugs. The cellulose ether hydroxypropylmethylcellulose has been widely applied as compressed hydrophilic matrix in which drug release occurred via a combination of diffusion and dissolution of the matrix itself following hydration [94]. The release of a number of drugs with varying hydrophilicity was investigated and near zero-order release was achieved for some water-soluble drugs such as diazepam [95]. Among several formulation parameters, varying the polymer concentration was found to be most efficient in controlling the drug release kinetics [96]. More recently, other natural polymers have been investigated as well for use in hydrogels, which will be shortly discussed in Section 5.4.

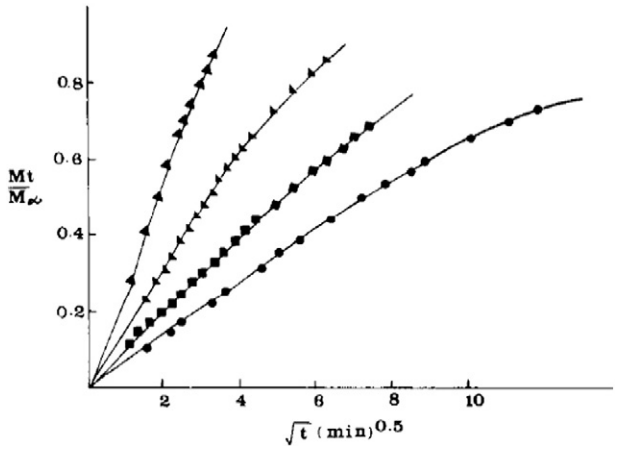

Fig. 5. Caffeine release from PEG hydrogels with constant length and width but varying thicknesses. ▲ 1.1 mm; ■ 2.0 mm; ▼ 3.7 mm; ● 5.0 mm.
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3. Second generation hydrogels

Inspired by the work of Katchalsky [97,98] in the 1950s and 1960s on the possibility of transferring chemical energy into mechanical work, in the beginning of the 1970s the hydrogel research focus shifted from relatively simple, water-swollen macromolecular networks to hydrogels capable of responding to a change in environmental conditions such as pH, temperature or concentration of biomolecules [99]. These environmental triggers can be used to evoke specific events, such as gel formation or drug release.

This section focuses mainly on temperature-sensitive hydrogels. Systems sensitive to pH or biomolecules will be addressed only shortly as these will be covered extensively by Siegel et al. elsewhere in this issue of the Journal of Controlled Release.

3.1. Temperature-sensitive hydrogels

The most widely studied environmentally responsive systems are temperature-sensitive hydrogels, in which physical entanglements, hydrogen bonding and hydrophobic interactions are the main features that constitute the crosslinks. The temperature dependent balance of these physical interactions governs the thermo-sensitive gelation behavior of the hydrogel. Temperature-sensitive hydrogels are of particular interest because they can be applied as *in situ* forming systems. These are fluids that can be injected into any tissue, organ or body cavity in a minimally invasive manner prior to gelation [100,101]. *In situ* forming hydrogels offer several advantages over systems that have to be formed into their final shape before implantation. To mention, there is no need for surgical procedures, biological components can easily be incorporated by simple mixing and their initially flowing nature ensures proper shape adaptation resulting in a good fit with the surrounding tissue. Poly(ethylene glycol)-polyester block copolymers, poly(N-isopropylacrylamide) (pNIPAAm) and poly(N-(2-hydroxypropyl)acrylamide) (PHPMAm) are the most extensively investigated polymers in this hydrogel class. Temperature-sensitive hydrogels are applied mainly for the controlled delivery of pharmaceutical agents.

3.1.1. Temperature-sensitive hydrogels based on PEG-polyester block copolymers

Aqueous solutions of selected poly(ethylene oxide)–poly(propylene oxide)–poly(ethylene oxide) (PEO–PPO–PEO) triblock copolymers, commercially known as Pluronics (BASF) or Poloxamers (ICI), exhibit a phase transition from the sol to the gel state at low temperatures and from the gel to the sol state at higher temperatures when the concentration is above the critical gel concentration (CGC). Pluronic hydrogels for the controlled release of pharmaceutical agents appeared in the 1970s. Early examples include Pluronic F127 gels for the controlled release of anesthetics [102] and antimicrobials [103]. More recently, the release of ophtalmics [104], cytostatics [105] and hormones [106] from Pluronic based hydrogels has been studied as well. Significant drawbacks of Pluronic hydrogels are their weak mechanical properties and intrinsic instability, which originate from the weak hydrophobic interactions between the PPO blocks. Moreover, these block copolymers are not biodegradable, which prevents the use of high molecular weight materials since they cannot pass the kidney membranes. These drawbacks prompted several researchers to replace the hydrophobic PPO block for a biodegradable polyester block as a basis for thermo-responsive hydrogels. Both AB-, ABA- and BAB-type

copolymers, with A as the PEG block and B the polyester block, were synthesized. Various polyesters have been employed as the hydrophobic block, predominantly poly(lactide) (PLA), poly(glycolic-co-lactic acid) (PLGA) and poly(ε-caprolactone) (PCL) because of their biocompatibility, biodegradability and facile synthesis via the ring opening polymerization of lactide, glycolide or ε-caprolactone. Most thermo-responsive hydrogels are based on PEG and PLA, and emphasis will be placed on these systems in the remainder of this section.

In the late 1990s, the group of Kim synthesized a number of linear AB diblock and ABA triblock copolymers, with A as a hydrophilic PEG block (M_n = 5 kg/mol) and B as a hydrophobic PLA block [107,108]. Diblock copolymers were synthesized by ring opening polymerization of lactide initiated by the hydroxyl group of monomethoxy PEG, while triblock copolymers were prepared by coupling the diblock copolymers with monomethoxy PEG using hexamethylene diisocyanate as chain extender. In comparison with diblock copolymers possessing the same PEG content and PEG molecular weight, triblock copolymers generally yielded hydrogels at lower polymer concentrations (Fig. 6). The thermo-responsive behavior can be tuned by the hydrophilic/hydrophobic balance, the block length, and the stereoregularity of the PLA block. For PEG–PLLA–PEG triblock copolymers, the CGC decreased from 20 to 12 w/v % upon increase of the PLLA block length from 2 to 5 kg/mol. Release studies were performed using fluorescein isothiocyanate labeled dextran (M_n 20 kg/mol) as model compound [109]. Zero-order release kinetics were observed for a 35 w/v % PEG–PLLA–PEG hydrogel, which released 40% of the initial drug load in 12 days. The release rate could be tailored by the initial loading, the molecular weight or hydrophobicity of the drug and the initial polymer concentration.

Block copolymers with an inverted structure (BAB) were prepared by ring opening polymerization of lactide initiated by the hydroxyl groups of PEG. At room temperature the CGC of triblock copolymers with a PEG M_n of 12.5 kg/mol decreased significantly from 80 to 15 w/v % upon an increase of the PLA block length from 10 to 15 lactyl units, showing a much stronger effect of the PLA block length on the gelation behavior in comparison with ABA-type triblock copolymers [110]. Li et al. investigated the degradation behavior of PLA–PEG–PLA triblock copolymers of high molecular weight (total M_n 45–75 kg/mol) [111]. Degradation was initially very fast with significant weight loss. The PLA/PEG ratio of the remaining material increased rapidly, indicating the release of PEG-rich chains. In the second phase, the degradation rate slowed down because of the high PLA content of the remaining material. The presence of proteinase K strongly accelerated the degradation rate of the hydrogels, showing that the enzyme was able to penetrate into the gel and attack the PLA domains. The PLA/PEG ratio in the residual hydrogel was found to increase as in the case of hydrolytic degradation.

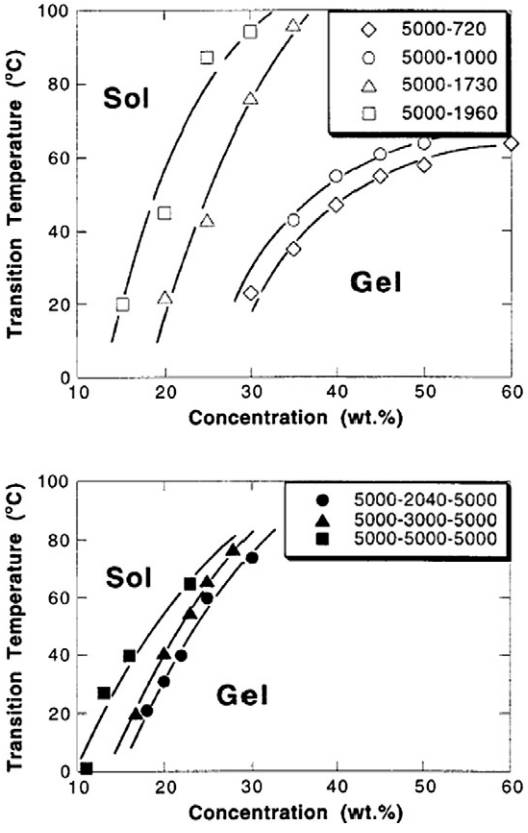

Fig. 6. Gel–sol transition curves of PEG–PLLA diblock (top) and PEG–PLLA–PEG triblock (bottom) copolymers. The numbers indicate the molecular weight of each block. Adapted from [108], copyright (1999), with permission from John Wiley & Sons, Inc.

Hydrogels based on alternating multiblock copolymers of PEG and PLA were also reported. The polymers were synthesized by coupling PEG-diols to PLA-diols using succinic anhydride [112,113] or by coupling hydroxyl end functionalized PLA–PEG–PLA triblock copolymers using adipoyl chloride [114]. The PEG/PLLA multiblock copolymers synthesized by the group of Jeong, having a total M_n of 7 kg/mol, exhibited a CGC of approximately 30 w/v % and underwent a sol–gel–sol transition with increasing temperature [112]. The gelation mechanism was considered by the authors to be governed by micellar aggregation. The transition temperature and gel modulus could be controlled by varying the PLLA block length and PEG molecular weight. The *in situ* gel forming ability of the polymers was demonstrated by subcutaneous injection into rats. The PEG/PLLA multiblock copolymer showed a lower CGC and improved mechanical properties in comparison with an analogous PEG/PDLLA multiblock copolymer [113], which was attributed to a lower dynamic molecular motion and a higher aggregation tendency of PLLA due to the isotactic localization of the hydrophobic methyl groups.

Besides the linear PEG–PLA copolymers, also a number of star-shaped and branched architectures were explored for the preparation of thermo-responsive hydrogels. Park et al. synthesized 3-armed PLA-centered star block copolymers by coupling monocarboxylated PEG to a 3-armed hydroxyl-terminated PLA using dicyclohexylcarbodiimide (DCC) as coupling agent [115]. At a similar PEG block length of 5 kg/mol, an increase in the PLA block length led to an expanded gelation window. The 3-armed star block copolymer exhibited a lower CGC in comparison with a PEG–PLA–PEG triblock copolymer possessing the same PEG content. Eight-armed PEG–PLA star block copolymer, prepared by ring opening polymerization of L-lactide using 8-armed PEG with hydroxy end-functional groups as an initiator, represents a star shaped BAB type copolymer [110]. These star block copolymers, with a PEG content of 74 wt.%, exhibited approximately the same gelation behavior as PLLA–PEG–PLLA triblock copolymers with a PEG content of 84 wt.%. Importantly, the CGC at room temperature decreased from 40 to 15 w/v % when the PLLA block length increased from 10 to 14 lactyl units. Increasing the PEG molecular weight at a constant PLLA block length also resulted in a lower CGC possibly due to enhanced chain entanglements. Recently, highly branched PEG–PLLA copolymers were synthesized by a coupling reaction of 8-armed amine-functionalized PEG and macromonomers having 2 PLLA arms and a N-hydroxysuccinimide activated ester group at the center of the polymer chain [116]. It was reported that 4 out of 8 PEG arms were functionalized with a branched PLA moiety. The copolymers showed thermo-responsive gelation behavior at low concentrations (4 w/v %). The gel–sol transition temperature could be tuned by varying the copolymer concentration and the molecular weight of the PLLA block. Branched block copolymers with a PLLA block length of 12 lactyl units exhibited significantly lower CGCs compared to the 8-armed PEG-PLLA star block copolymers with a similar PEG content and a PLLA block length of 10 lactyl units [110]. This drop in CGC was

ascribed to stronger hydrophobic interactions in the branched system, because hydrophobic domains may be formed more easily if only 4 out of 8 arms have to be folded into such a domain instead of 8 out of 8 arms (Fig. 7). Recently it was found that 8-armed PEG-PLA star block copolymers, possessing an amide linkage between PEG and PLA, yielded hydrogels with improved mechanical properties and a controlled hydrolytic degradation compared to 8-armed PEG–PLA star block copolymers having an ester linkage between the PEG and PLA blocks [117,118].

Hydrogels based on PEG–PLGA–PEG triblock copolymers were introduced in the late 1990s as an alternative for early PEG–PLA based systems. These systems exhibit a sol-to-gel transition at lower temperatures and a gel-to sol transition at higher temperatures. High temperatures, which are potentially harmful for bioactive molecules, can consequently be avoided for the preparation of PEG–PLGA–PEG hydrogels [120]. In PEG–PLGA–PEG systems with a PEG block of <750 g/mol, the gel window covered the physiological temperature and the CGC and sol-to-gel transition temperature could be tuned by the block length and composition as well as by the addition of additives [121]. The lower sol-to-gel transition was ascribed to growth, packing and interaction of the micelles, while the upper gel-to-sol transition was thought to be due to the collapse of the micellar structure after dehydration of the PEG block [122,123]. After subcutaneous injection of an aqueous solution of PEG–PLGA–PEG (550–2810–550 g/mol) into rats, a transparent hydrogel was formed *in situ* which exhibited good mechanical properties and was stable for 1 month [124]. PEG–PLGA–PEG based hydrogels have been used for the controlled release of various bioactive molecules, including the model drugs ketoprofen and spironolactone [125], and the growth factor TGF-β1 [126].

BAB-type PLGA–PEG–PLGA triblock copolymers were subsequently introduced as new hydrogel forming materials and commercialized as ReGel [127]. These polymers were synthesized by ring opening polymerization of DL-lactide and glycolide initiated by PEG, without the need for a possibly toxic coupling agent such as hexamethylene diisocyanate in the case of ABA triblock copolymers. PLGA–PEG–PLGA showed a lower CGC and sol-to-gel transition temperature in comparison with PEG–PLGA–PEG, suggesting a different gelation mechanism for BAB type block copolymers. The temperature-dependent ordered packing of bridged micelles was suggested as the gelation mechanism of concentrated PLGA–PEG–PLGA aqueous solutions [128]. *In vitro* release experiments showed a diffusion-controlled release of paclitaxel from ReGel in the first two weeks, followed by release via a combination of diffusion and hydrogel degradation for 5 weeks [129]. In contrast, paclitaxel release from a Pluronic hydrogel was complete within 1 day (Fig. 8). *In vivo*, paclitaxel-loaded ReGel showed a higher tumor efficacy and fewer adverse effects than the clinically used formulation of paclitaxel (Taxol). ReGel formulations have also been employed for the controlled release of proteins such as interleukin-2 [130], insulin [131] and testosterone [132].

Bae et al. prepared ABA and BAB type block copolymers based on PCL and PEG [133,134]. Copolymers with an appropriate hydrophilic/hydrophobic balance showed both a lower sol-to-gel transition and an upper gel-to-sol transition with increasing temperature, which were ascribed to micellar aggregation through hydrophobic interactions and micellar collapse through increased molecular motion of PCL, respectively. Similar to PL(G)A/PEG based systems, PEG and polyester block lengths as well as block topography were found to influence gelation properties. Solutions of PCL–PEG–PCL exhibited a lower critical gelation temperature and a larger gel window compared with PEG–PCL–PEG, probably due to the possibility of intermicellar PCL bridging leading to more facile aggregation. The release of the model drugs vitamin K, honokiol and albumin from PCL–PEG–PCL hydrogels was governed mainly by diffusion as opposed to matrix degradation because of the high *in vitro* hydrolytic stability of the PCL blocks [135].

Recently, temperature-responsive gelling systems composed of poly(ε-caprolactone-co-lactide)–b-poly(ethylene glycol)–b-poly(ε-caprolactone-co-lactide) (PCLA–PEG–PCLA) triblock copolymers were developed [136,137]. It was demonstrated that the molecular weight of PCLA, the caproyl/lactoyl ratio and the nature of the end group had a profound effect on the crystallinity and consequently on the rheological and degradation properties. Good cytocompatibility of an acetyl-capped PCLA–PEG–PCLA hydrogel was proven *in vitro* on erythrocytes and chondrocytes. Moreover, intra-articular biocompatibility *in vivo* was demonstrated using microCT-imaging and histology, as both techniques showed no changes in cartilage quality and/or quantity [138].

Other examples of hydrophobic blocks in amphiphilic PEG copolymer hydrogels intended for biomedical applications include poly(3-methylglycolide) [139], poly(δ-valerolactone) [140], poly(propylene fumarate) [141] and poly(trimethylene carbonate) [142].

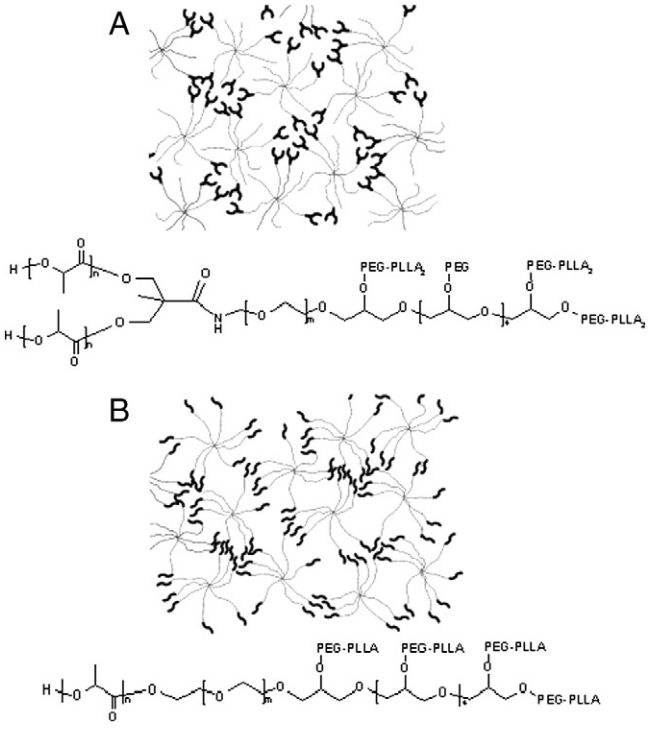

Fig. 7. Representation of a hydrogel prepared with the highly branched PEG–PLA block copolymer described by Velthoen et al. (A) [116] and the 8-armed PEG–PLA star block copolymer described by Hiemstra et al. (B) [119].
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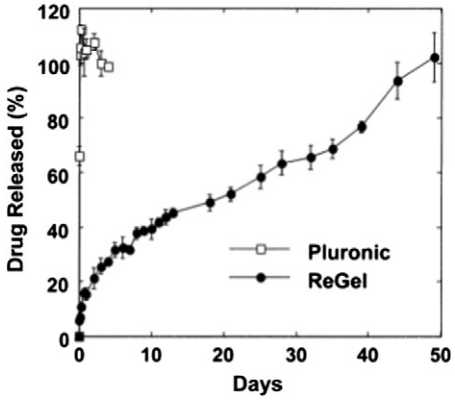

Fig. 8. *In vitro* release of paclitaxel from PLGA–PEG–PLGA and Pluronic hydrogels.
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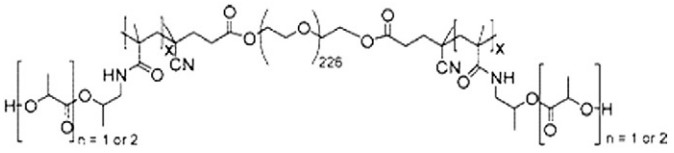

Fig. 9. Chemical structure of ABA triblock copolymers consisting of a PEG middle block and random outer blocks of HPMA mono and di lactate.

3.1.2. Temperature-sensitive hydrogels based on pNIPAAm

Although poly(N-isopropylacrylamide) (pNIPAAm) was first synthesized in the 1950s as a rodent repellent [143], it did not gain much attention until in 1968 Heskins and Guillet reported about the temperature dependent phase transition of pNIPAAm solutions in water [144]. They ascribed the lower critical solution temperature (LCST) of these solutions to preferred intermolecular hydrogen bond formation between pNIPAAm chains over water-amide hydrogen bond formation due to an entropy effect. Since then, many researchers made attempts to fully elucidate the mechanism of pNIPAAm's thermal behavior [143,145,146]. Using techniques such as light scattering and viscometry, they found a sharp and reversible phase transition of pNIPAAm around 32 °C, almost independent of molecular weight and concentration [147,148]. Given the fact that this phase transition occurs between room temperature and body temperature, interest sparked for using pNIPAAm as an injectable material for biomedical applications. However, at high concentrations the phase transition from a hydrated swollen state to a collapsed dehydrated state resulted in a loss of 90% of water [143]. This phenomenon, also known as syneresis, hindered the initial application of pNIPAAm in hydrogel formulations. Therefore, pNIPAAm was copolymerized with a variety of monomers to introduce new functionalities and to prevent syneresis. Copolymerization with a monomer such as dihydroxyethylene-bis-acrylamide and subsequent radical polymerization resulted in the formation of a covalent network besides the thermally induced physical network [149]. Since the 1980s, crosslinked pNIPAAm hydrogels were studied for among others the release of vitamin B12, myoglobin [150] and progesterone [151]. However, the release of drugs from these hydrogels was relatively fast, usually within 24 h. To extend the release time and to enhance the mechanical stability, interpenetrating polymer networks of pNIPAAm were formed [152]. Alternatively, pNIPAAm hydrogels were covalently crosslinked with another polymer with complementary reactive groups. The combination of temperature induced physical crosslinking and chemical crosslinking will be discussed in Section 5.3. Additionally, pNIPAAm was copolymerized with hydrophilic or hydrophobic monomers to either increase or decrease its LCST [153]. Despite all these unique properties, pNIPAAm is a non-degradable polymer, therefore limiting its application for drug delivery and tissue engineering purposes [154]. Introduction of biodegradable segments in pNIPAAm resulted in the formation of toxic low-molecular mass pNIPAAm degradation products [155,156]. For this reason, the most attractive approach to form a bioresorbable hydrogel based on pNIPAAm is by manipulating its LCST in time. Hennink and coworkers have developed copolymers of NIPAAm and 2-hydroxyethyl methacrylate monolactate (HEMA-lactate) [157]. During incubation in an aqueous solution the lactate ester side groups are hydrolyzed. Due to this hydrolysis, the overall hydrophilicity increased, resulting in an LCST above 37 °C and therefore dissolution of the polymer. The LCST could be tuned depending on the length of the lactate side groups and in this way a high control over degradation time was achieved [158]. Later on, HEMA was replaced by N-(2-hydroxypropyl) methacrylamide (HPMA) for its hydrophilicity and low immunogenicity [159,160]. With variations in the formulation a highly tunable degradation rate was obtained [161]. Using a similar hydrolysis-sensitive approach, pNIPAAm has also been copolymerized with dimethyl-γ-butyrolactone acrylate (DBA) that initially resulted in a decrease in LCST with increasing DBA content [162]. In time hydrolysis resulted in an increase of the LCST above 37 °C.

3.1.3. Other thermoresponsive systems

Thermoresponsive, hydrolytically degradable polymers have also been designed solely based on HPMA-lactate monomers. ABA triblock copolymers with thermosensitive poly(N-(2-hydroxypropyl) methacrylamide lactate) A-blocks and a hydrophilic PEG B-block, which can form hydrogels at 37 °C have been synthesized by our group (Fig. 9) [163]. Extensive rheological studies showed a high tunability in storage modulus by changing the concentration of the polymers and thermosensitive block length. Diffusion of fluorescein isothiocyanate (FITC)-labeled dextran in these hydrogels was described with a rate depending on polymer design, concentration and temperature [164].

Lutz and Hoth described the synthesis of copolymers of 2-(2-methoxyethoxy)ethyl methacrylate and oligo(ethylene glycol) methacrylate (P(MEO_2MA-co-OEGMA), also abbreviated as pOEGMA) [165]. These polymers were synthesized by atom transfer radical polymerization (ATRP) and displayed LCST behavior in water. These systems were particularly of interest since the LCST of these polymers could be precisely tuned depending on the feed ratio of the two monomers [165]. Additionally, these scientists compared these polymers with pNIPAAm and found a similar independence of the LCST on pH and polymer concentration [166]. Moreover, advantages of pOEGMA over pNIPAAm include a smaller difference between phase transition temperature during heating and cooling cycles, a smaller dependence of the LCST on the polymer chain length (Fig. 10) and a suggested higher biocompatibility [166]. POEGMA based microgels have for example been used for delivery of chemotherapeutic agents [167].

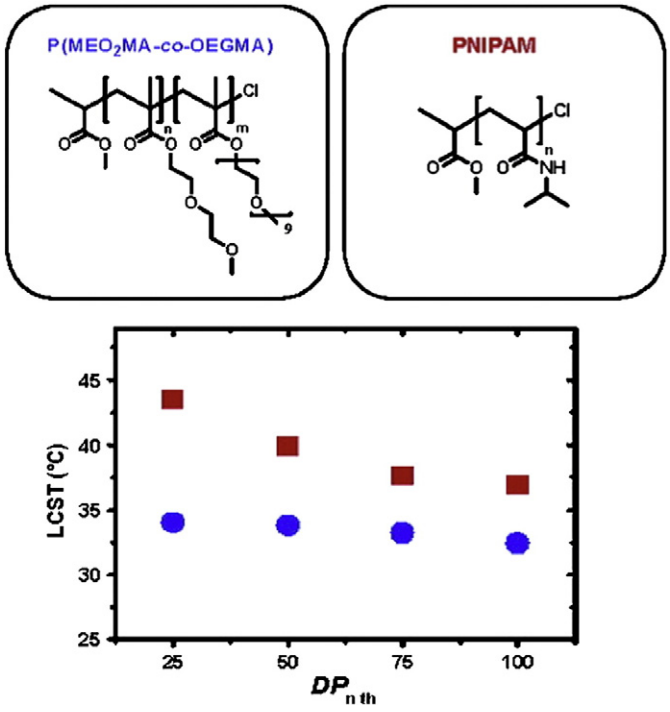

Fig. 10. Chemical structures of pOEGMA and pNIPAAm. The graph shows the effect on the LCST for different theoretical degrees of polymerization (DP_{nth}) for pOEGMA (red squares) and pNIPAAm (blue circles).
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3.2. In situ forming hydrogels based on other stimuli

Besides temperature-sensitive hydrogels, the second most used trigger for hydrogel formation is pH. Generally, pH sensitive hydrogels contain either basic or acidic moieties that become ionized at high or low pH, respectively [168,169]. Making use of the variation in pH in different parts of the body, controlled release of the hydrogel content can be established.

Lastly polymers that respond to concentrations of biomolecules have attracted interest as *in situ* forming materials. Biomolecule-sensitive hydrogels can be regarded as perfect mimics of nature, since a conformational change is induced after change in concentration of biomolecules, like the body responds on hormones [170]. The most well-known biomolecule in this category is glucose. Hydrogels were designed containing glucose oxidase, together with pH sensitive moieties. After glucose diffuses into these hydrogels, it is converted to gluconic acid, leading to a decrease in pH and subsequently increase in swelling of the hydrogel due to e.g. protonation of amine functionalities present in the network [171,172]. Insulin can then be released from the hydrogel, showing the potential of these hydrogels as self-regulating systems. A more detailed overview of pH and biomolecule sensitive hydrogels will be given by Siegel et al. elsewhere in this journal issue.

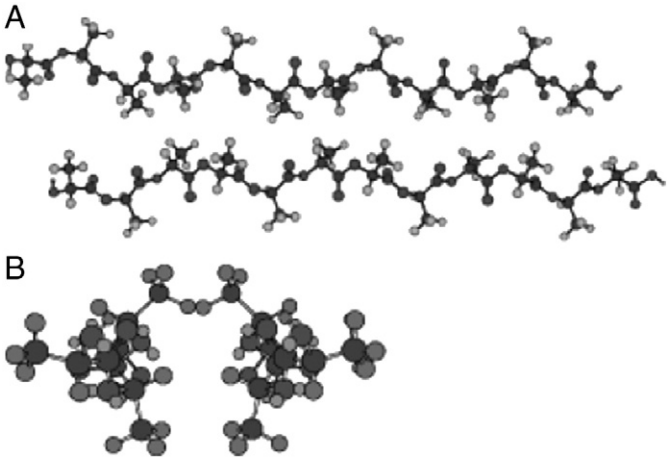

Fig. 11. (A) Projections along the helical axis of a helical conformation of stereocomplexed PLA (upper: PLLA, lower: PDLA). (B) Projections perpendicular to the helical axis of helical conformations of PLLA (left) and PDLA (right) in stereocomplexed PLA.
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4. Third generation hydrogels

The temperature- and pH-responsive physical hydrogels discussed in the previous chapter are mainly crosslinked via hydrophobic and ionic interactions, respectively. In the mid-1990s, other physical interactions were recognized and exploited as crosslinking methods that offered the possibility to enhance and finely tune the mechanical, thermal and degradation properties of hydrogels. Many of these interactions also allowed for *in situ* hydrogel formation. This section focuses on stereocomplexation, inclusion complex formation, metal–ligand coordination and peptide interactions as crosslink methods for the preparation of hydrogels.

4.1. Stereocomplexed hydrogels

A polymer stereocomplex is defined as a stereoselective interaction between two complementing stereoregular polymers, which interlock and form a new composite with altered physical properties in comparison with the constituting polymers [173]. The first example of stereocomplexation was reported in 1953 by Pauling and Corey for a polypeptide [174]. Stereocomplexation between enantiomeric polylactides was first noticed by Ikada et al. in 1987 [175]. The complementary polymers PLLA and PDLA are optically active polymers with identical chemical structures but opposite chirality. In the solid state, PLLA forms a left-handed helix, while PDLA forms a right-handed helix. It has been suggested that van der Waals forces between the two helices are the driving force for a dense crystalline packing of the helices in a stereocomplex (Fig. 11) [176,177]. Because stereocomplex crystals are formed at shorter PLA block lengths compared to homopolymer crystals [178], an operation window exists in which mixing of aqueous solutions of PLLA and PDLA block copolymers results in the formation of a hydrogel through crosslinking by stereocomplexation. From 2000 onwards, stereocomplexation between enantiomeric PLLA and PDLA blocks in amphiphilic copolymers has been employed for the preparation of injectable hydrogels.

Kimura and coworkers investigated the influence of the architecture of stereocomplexed PEG–PLA block copolymers on the gelation properties [179,180]. Ring opening polymerization of L- or D-lactide initiated by mono- or dihydroxy PEG generated enantiomeric AB diblock and BAB triblock copolymers, respectively, whereas ABA triblock copolymers were obtained by coupling the AB diblock copolymers with hexamethylene diisocyanate. The PEG content of all copolymers in these studies was approximately 50 wt.%. Whereas BAB type copolymers may show thermo-reversible gelation, mixing of aqueous solutions of enantiomeric BAB block copolymers afforded systems that exhibit an irreversible sol–gel transition upon temperature increase.

Triblock copolymers with an ABA structure, on the other hand, formed hydrogels at high concentrations showing a reversible gel–sol transition with temperature. Mixed solutions of enantiomeric AB diblock copolymers also yielded hydrogels that exhibit a gel–sol transition upon temperature increase. However, unlike the ABA triblock system, this transition is irreversible.

Grijpma et al. and Li et al. reported on the synthesis, characterization and stereocomplex mediated gelation of PEG–PLA diblock and PLA–PEG–PLA (BAB) triblock copolymers [181–184]. Thymopentin release from these systems was slower in comparison with the release from single enantiomer hydrogels [185]. When the copolymers were synthesized by ring opening polymerization of L- or D-lactide initiated by dihydroxy PEG (M_n 4 kg/mol) and zinc lactate as a catalyst for 7 days, hydrogel formation by stereocomplexation was detected for copolymers with PLA blocks of 17 lactyl units but not for copolymers with PLA blocks of 11–13 lactyl units [186]. This was ascribed to racemization of L-lactyl units leading to non-isotactic sequences in the PLLA chains, which prevents the formation of stereocomplexes. Racemization was largely reduced when the reaction time was shortened to 1 day. It appeared that 10 lactyl units per PLA block were sufficient for the formation of stereocomplexed PLA–PEG–PLA hydrogels.

Star-shaped block copolymers of PEG and PLA, showing stereocomplex mediated gelation, were also investigated. It was found that stereocomplexed PEG–$(PLA)_8$ star block copolymers, prepared by ring opening polymerization of L- or D-lactide initiated by 8-armed PEG (M_n 20 kg/mol), gelated faster and formed hydrogels with improved mechanical strength as compared to gels based on stereocomplexed PLA–PEG–PLA triblock copolymers [110]. This was ascribed to a higher number of stereocomplex sites in PEG–$(PLA)_8$ [119]. Rheological measurements showed that increasing the average PLA block length from 12 to 14 lactyl units at a polymer concentration of 10 w/v % resulted in an increase in the storage modulus from 0.9 to 7.0 kPa and a decrease in gelation time from 40 min to less than 1 min. It was shown that the release of the relatively small protein lysozyme followed first order kinetics and approximately 90% was released in 10 days [187]. The relatively large protein immunoglobulin G was released from stereocomplexed hydrogels with nearly zero order kinetics, and up to 50% was released in 16 days. Replacing the ester linkages between the PEG core and the PLA arms by amide linkages resulted

in stereocomplexed hydrogels with improved mechanical properties and controlled degradation at 37 °C in PBS [188,189].

Nagahama et al. prepared enantiomeric 8-armed PEG–PLA–PEG type copolymers by coupling monocarboxylated PEG to star-shaped PEG–PLLA or PEG–PDLA diblock copolymers using N,N′-dicyclohexylcarbodiimide as a coupling agent [190]. At low concentrations an aqueous mixture consisting of both enantiomers yielded a sol at room temperature exhibiting an irreversible transition to the gel state upon temperature increase. *In vitro* degradation experiments revealed a faster molecular weight reduction for copolymers in single enantiomer hydrogels compared to mixed enantiomer hydrogels. This suggests that stereocomplex formation has an inhibitory effect on the hydrolysis of the ester groups in the PLA domains.

Also polymers other than PEG have been used in combination with PDLA and PLLA to prepare stereocomplexed hydrogels. Hennink and coworkers synthesized hydrogels from the natural polysaccharide dextran grafted with monodisperse L-lactic acid and D-lactic acid oligomers [191]. Rheological experiments showed that the degree of polymerization (DP) of the lactic acid oligomers must be at least 11 to obtain a hydrogel. Stronger gels were obtained by increasing the DP and degree of substitution and by decreasing the water content. The degradation time varied from 1 to 7 days, depending on the number, length and polydispersity of the lactate grafts and the initial water content [192]. Protein-loaded hydrogels were prepared by dissolving the proteins in the enantiomeric dextran–lactate solutions prior to mixing. Lysozyme was released in 5 days by diffusion, whereas the release of the larger immunoglobulin G in 8 days was governed by diffusion as well as swelling and degradation of the hydrogel. *In vivo* tests demonstrated that these stereocomplexed hydrogels are biocompatible and effective systems for the local delivery of the cytokine recombinant human interleukin-2 [193,194]. In an alternative approach, it was shown that a mixture of crosslinked dextran microspheres substituted with L- or D-oligolactates also resulted in macroscopic hydrogels with high stiffness. Protein release experiments with these systems revealed a continuous lysozyme release during 30 days with full preservation of its enzymatic activity [195].

Van Nostrum et al. reported on stereocomplexed hydrogels prepared from pHPMAm with oligo(lactic acid) side chains of opposite chirality [196]. In comparison with dextran stereocomplexed hydrogels, the degradation time of the pHPMAm stereocomplexed hydrogels was significantly longer, mainly due to the presence of carbonate instead of ester linkages between the oligolactide and the polymer backbone. The degradation time could be tailored from 1 week to 3 months by changing the grafting density and the oligolactide endgroup.

Stereocomplexed hydrogels were also prepared from oligolactide-functionalized pHEMA [197], Pluronics [198] and 2-methacryroyloxyethyl phosphorylcholine [199] polymers. An extensive recent review concerning stereocomplexes in biomedical applications can be found in reference [200].

4.2. Hydrogels crosslinked by other physical interactions

Cyclodextrins (CDs) are cyclic oligosaccharides possessing a hydrophobic cavity that can act as a host for a variety of molecules. The formation of a CD-polypseudorotaxane, where a polymeric guest threads into multiple CD hosts, and subsequent polypseudorotaxane aggregation can lead to the formation of a supramolecular hydrogel (Fig. 12). This hierarchical self-assembly process was employed for the first time to create a physically crosslinked hydrogel by Li et al. in 1994 [201]. Disadvantages of early systems, which were based on PEG as guest molecule, included low stability and long gelation times of several hours. Improved hydrogel properties were obtained when aqueous solutions of CDs were mixed with amphiphilic block copolymers such as Pluronics [202], reverse Pluronics (PPO–PEO–PPO) [203], PCL–PEG–PCL [204] and poly(ethylene glycol)-poly(3-hydroxybutyrate)-poly(ethylene glycol) (PEG–PHB–PEG) [205]. For example, a 23 wt.% hydrogel obtained

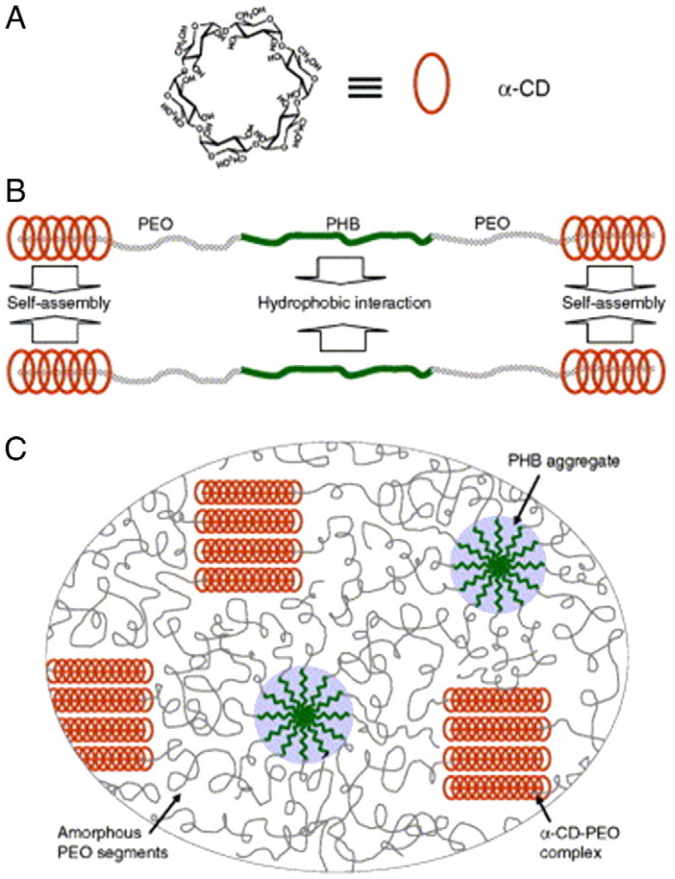

Fig. 12. The structure of α-CD (a), the schematic illustrations of the proposed structures of α-CD–PEO–PHB–PEO inclusion complex (b), and α-CD–PEO–PHB–PEO supramolecular hydrogel (c).
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from a PEG–PHB–PEG amphiphilic triblock copolymer (M_n 13 kg/mol) and α-CD released fluorescein isothiocyanate labeled dextran for over 30 days, in contrast to a similar formulation prepared from high molecular weight PEG (M_n 35 kg/mol) which was only stable for 6 days [206]. CD hydrogels were also prepared with a number of PEG-grafted natural polymers, including dextran [207], chitosan [208] and heparin [209]. In these cases, the high degree of crosslinking due to the branched polymer architecture resulted in stiff and stable hydrogels. The supramolecular heparin hydrogel described by Zhang et al. showed a sustained release of the model protein albumin, which could be tuned by the heparin and α-CD concentration. Interestingly, this supramolecular hydrogel also exhibited a controlled release profile for heparin, and good anticoagulant and blood-compatible properties were reported [209]. A recent development in this field is the preparation of stimuli-responsive polypseudorotaxane hydrogels, including pH- [210], photo- and dual-responsive [211] systems.

Another widely explored strategy to produce hydrogels is the co-assembly of CDs with hydrophobic groups grafted on polymers. These developments occurred simultaneously with the emergence of the polypseudorotaxane hydrogels described above. Especially the adamantane group has been extensively used a guest molecule for complexation with CD. For example, a thermo-responsive hydrogel was described based on a combination of adamantyl end-functionalized PEG and star-shaped pNIPAAM comprising a β-CD core [212]. Van de Manakker et al. prepared hydrogels from a combination of two complementary 8-armed star-shaped PEGs, end-functionalized either with β-CD or with cholesterol [213,214]. The gel properties could be tuned via the polymer concentration, molar

ratio of end groups, molecular weight, architecture of the PEG, temperature and addition of a competitive inclusion complexing agent. Degradation occurred via surface erosion, resulting in near zero-order release of the model protein lysozyme, albumin and immunoglobulin G [215]. When 8-armed cholesterol-functionalized PEG was combined with free β-CD, hydrogels with improved mechanical properties were obtained [216]. Various other hydrogels based on CD inclusion complexes have been described and reviewed elsewhere [217–219].

Only a few reports on metallohydrogels, in which the reversible bonds between macromonomers are based on metal–ligand coordination, have been published. The earliest examples were reported by Chujo et al., who prepared hydrogels based on bipyridine-functionalized poly(oxazoline) crosslinked by Co(II) [220] or Fe(III) [221] ions. The gels kept their integrity at ambient temperatures for several days, but dissolved rapidly upon temperature increase due to a shift from intermolecular coordination complexes to the entropically favored intramolecular coordination complexes. Later PEG [222], Pluronics [223] and PEG–PLA [224] end-functionalized with ligands such as terpyridine or bipyridine were employed as well to prepare hydrogels in the presence of transition metal ions such as Mn(II) and Ni(II). Recently, PEG-based hydrogels were synthesized via coordination between metal ions and bisphosphonate [225] or histidine groups [226].

The self-assembly of natural building blocks such as peptides into ordered structures, mainly coiled coils or β-sheets, has also been employed for the preparation of hydrogels. Two approaches were used for the design of macromolecules that self-assemble into hydrogels: genetically engineered copolymers and hybrid systems composed of a synthetic polymer and a peptide or protein motif. In the first category, Tirrell et al. performed pioneering work on the self-assembly of genetically engineered triblock copolymers prepared from a random coil block flanked by 2 coiled-coil forming blocks [227]. Self-assembly into hydrogels resulted from the balance between oligomerization of the helical ends and swelling of the central water soluble segment. Temperature- and pH-sensitivity could be manipulated by altering the amino acid sequence in the coiled-coil domain [228]. Cappello et al. prepared protein polymers composed of tandemly arranged silk-like blocks and elastin-like blocks [229–231]. The silk-like blocks form crystallizable hydrogen-bonded β-sheets, providing the hydrogels with thermal and chemical stability, whereas the elastin-like blocks decrease the crystallinity and increase the water solubility of the copolymers. Plasmid DNA delivered from a silk-elastin protein hydrogel showed up to 3 orders of magnitude higher transfection in a murine model of human breast cancer in comparison with naked DNA [232]. Hydrogels prepared from poly(amino acid) diblock and triblock copolymers were investigated by Deming et al. [233]. Block copolymers composed of poly(L-lysine) or poly(L-glutamic acid) as hydrophilic block and poly(L-leucine) or poly(L-valine) as the hydrophobic block formed hydrogels at concentrations as low as 0.25 wt.%.

The first example of a hybrid hydrogel crosslinked via assembly of coiled coils was reported by the group of Kopecek. They used a HPMA copolymer backbone which was non-covalently crosslinked by a genetically engineered coiled coil protein motif [234]. A non-reversible temperature-induced hydrogel collapse was observed corresponding to the structural transition of the coiled-coil domains from an elongated helix to an unfolded state. It was shown that reversibility of the gel–sol transition can be achieved by crosslinking the HPMA with a pair of oppositely charged peptide grafts [235]. Recently, hydrogels from HPMA polymers with complementary β-sheet peptide grafts were explored for bone tissue engineering [236]. It was demonstrated that the hydrogels provide a support for pre-osteoblast cells and for the template-driven mineralization of hydroxyapatite. Hybrid hydrogels based on PEG coupled to coiled-coil (Fig. 13) [237] or β-sheet [238] forming peptides were also reported.

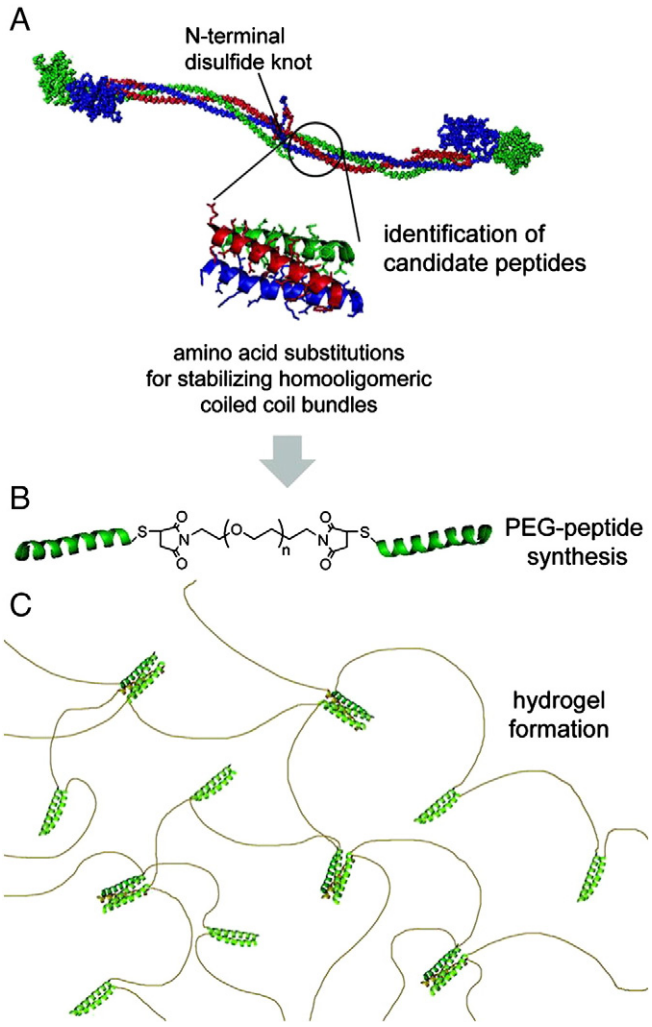

Fig. 13. Strategy for the preparation of fibrin-inspired coiled-coil biomaterials. Short peptides from the coiled-coil domain of fibrin are identified (a). Substitutions are made to stabilize homo-oligomeric coiled-coil formation, and designed peptides are conjugated to short PEG chains to form triblock peptide–PEG–peptides (b). Triblock copolymers self-assemble in appropriate buffers to produce hydrogels (c).
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A new development is the design of hydrogels capable of translating a specific stimulus, such as enzyme–substrate recognition [239], into macroscopic motion. The field of self-assembling hybrid hydrogels was extensively reviewed by Kopecek in a recent publication [240].

5. Smart hydrogels

With the increasing knowledge in organic chemistry, a variety of chemically crosslinked hydrogels has been developed. This section describes the use of smart hydrogels i.e. *in situ* formation of hydrogels through covalent crosslinking between polymers with complementary functional groups that occurs under physiological conditions with minimal toxicity. Additionally, double network hydrogels with a combination of physical, covalent or ionic bonds are described. Further, this section discusses the design of multi-component hydrogels capable of responding to multiple triggers or forming mechanically strong hydrogels. Altogether, these smart hydrogels allow tailoring properties such as mechanical stability and release kinetics for the desired application.

5.1. In situ chemically crosslinkable hydrogels

Hydrogel formation can be catalyzed by enzymes such as horseradish peroxidase [241], transglutaminase [242,243] and tyrosinase [243]. This class of hydrogels has mostly been applied for tissue engineering and as adhesive materials [13]. However, the use of enzymatic crosslinkable hydrogels is mainly limited by the stability of some enzymes.

Hydrogels formed from polymers with complementary functional groups offer a great tunability in hydrogel properties, such as gelation kinetics and mechanical stability [14,244]. In contrast to small-molecule crosslinkers, toxicity of these systems is often limited. Especially polymers that can crosslink under physiological conditions are interesting for drug delivery applications. For example amine and aldehyde-functionalized hyaluronic acid precursors have been synthesized with different degrees of substitution [245]. Mixing of these two precursors in buffer resulted in the formation of a hydrogel. Other examples of chemical crosslinking reactions to form hydrogels include Passerini and Ugi condensation [246,247] and disulfide formation [248]. In 2002, our group published an extensive overview of different chemical crosslinking reactions for the formation of hydrogels [14]. Therefore, this section will focus only on the recent efforts in *in situ* forming hydrogels without the need of catalysts and hydrogel formation by chemoselective crosslinking strategies. Michael addition, a conjugation reaction between nucleophiles such as thiols and electrophilic olefins e.g. (meth)acrylates, has been widely applied for the formation of hydrogels. In 2001 the group of Hubbell described the formation of a hydrogel based on PEG–multiacrylate and PEG dithiol precursors [249]. Albumin was released from these hydrogels with zero-order kinetics and with a rate depending on the polymer concentration. Michael addition is particularly attractive as *in situ* chemical crosslinking method due to its relatively fast kinetics and reaction under physiological conditions without the need for catalysts. For example, PEG–dextran [250], PEG–oligopeptide [251], PEG–PLA [252] and PEG–hyaluronic acid [253] hydrogels have been prepared using Michael-type addition. Recently, emphasis on controlled crosslinking of hydrogels *in situ* under physiological conditions, without toxic additives has resulted in the use of chemoselective crosslinking strategies for hydrogel formation. Chemoselective crosslinking has the benefit to not interfere with biomolecules such as proteins. The most researched chemoselective crosslinking reaction for hydrogels is click chemistry [85,254,255]. Click chemistry was first introduced by Sharpless and coworkers as new regiospecific linking reactions that give high yields and generally require no purification [256]. Among the different reactions, Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition of terminal alkynes and azides is the most investigated method [257]. Since click reactions can take place under physiological conditions with fast kinetics, it received great attention in pharmaceutical sciences [255,257,258]. The first hydrogel designed by click chemistry was based on poly(vinyl alcohol) and described by Ossipov and Hilborn [259]. Hydrogels crosslinked by click chemistry have for example been used for the release of doxorubicin and benzidamine using hyaluronic acid precursors [260]. However, alkyne-azide cycloadditions generally require the use of toxic catalysts, such as copper, limiting their application in the biomedical field. Recently, hydrogels have been crosslinked by copper-free click chemistry using strain promoted precursors, thereby eliminating the need of (metal-ion) catalysts [261–263]. As is shown in Fig. 14, covalently crosslinked, degradable hyaluronic acid hydrogels were prepared using azide and cyclooctyne functionalized precursors.

More recently, native chemical ligation has been investigated as an alternative chemoselective crosslinking reaction for the formation of hydrogels [86,264]. In native chemical ligation an N-terminal cysteine and thioester react to form a native peptide bond. This reaction has mostly been applied for the synthesis of peptides and proteins [265]. Cells were successfully incorporated in these hydrogels and were capable of forming new extracellular matrix [266,267]. Using a related reaction mechanism, hydrogels were also formed by oxo-ester mediated native chemical ligation [268]. This reaction has the advantage to increase the reaction kinetics and capture thiol reaction products, resulting in increased cell viability.

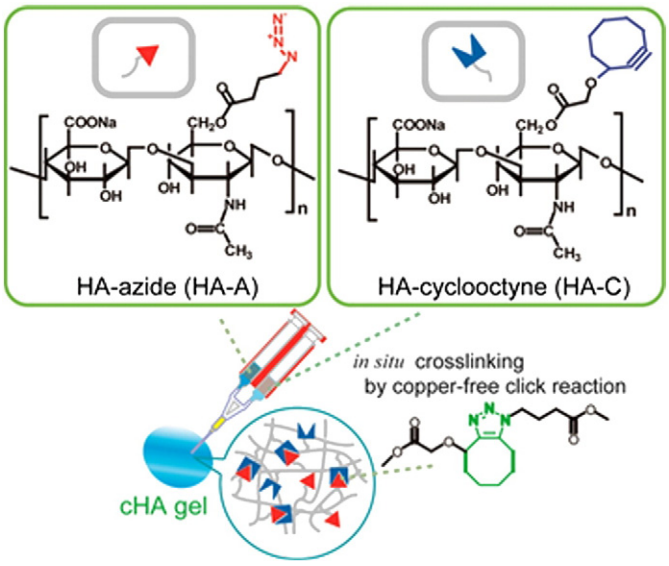

Fig. 14. *In situ* copper-free click reaction using strain promoted hyaluronic acid precursors. Reprinted with permission from [263]. Copyright (2013) American Chemical Society.

5.2. Radical polymerization

Macromers containing methacrylate or acrylate moieties can be crosslinked via radical polymerization using potassium persulfate (KPS) and N,N,N′,N′-tetramethylethylenediamine (TEMED) as initiator and catalyst respectively. Hydrogels formed by a redox reaction with TEMED/KPS were first described by Saito et al. in 1990 [269,270]. Our group prepared dextran-hydroxy-ethyl-methacrylate (dex-HEMA) hydrogels using this redox reaction, where the mechanical properties could be tuned depending on KPS concentration and temperature [271]. Further, controlled release of lysozyme, albumin and IgG from these hydrogels was demonstrated [272]. Similarly, our group described the formation of hydrogels from methacrylated hyaluronic acid [273]. A high control over degree of methacrylation allowed the design of hydrogels with a variety of mechanical properties. Kasper et al. reported about the ammonium persulfate/TEMED mediated crosslinking of oligo(poly(ethylene glycol)fumarate) (OPF) hydrogels for the controlled release of plasmid DNA, with a release rate depending on the OPF molecular weight [274]. Recently, hydrogels formed by radical polymerization of poly(N-isopropylacrylamide) and *N,N*-methylenebisacrylamide have been designed containing bridged nanogels and consequently showing surprisingly high elasticity and mechanical resistance [275]. However, unreacted persulfate and TEMED, including their degradation products, can oxidize and thereby damage encapsulated proteins. Therefore, only after complete removal of these compounds, the hydrogels can be used for biomedical applications [276].

In a different approach, network formation between (meth)acrylate functionalized polymers can occur in the presence of UV or visible light [277,278]. The photopolymerization reaction is initiated by decomposition of a photoinitiator, resulting in the formation of radicals and subsequent network formation. The formation of a hydrogel through photopolymerization has been first described by Hubbell et al. in 1993 and the formed gels released BSA for two months [279]. Later, they described the release of several other proteins and oligonucleotides from these acrylate-modified PEG-oligo-(α-hydroxy acids) hydrogels [280].

Due to the relative ease of introducing (meth)acrylate functionalities to polymer precursors, recently a variety of natural polymers has been chemically crosslinked after photopolymerization. A multi-component hydrogel consisting of photo-curable gelatin, hyaluronic acid and chondroitin showed capable of facilitating the production of extracellular matrix for cartilage regeneration [281].

Photo-curing hydrogels have several advantages over other *in situ* forming materials. The network formation is fast and the illumination allows a high spatiotemporal control [277]. Additionally, precise illumination can allow the formation of patterned hydrogels and non-uniform concentration profiles, resulting in different drug release profiles. This was demonstrated by Lu et al. with the release of an organic dye from crosslinked poly(2-hydroxyethyl methacrylate) (poly(HEMA)) hydrogels [282,283]. Some drawbacks of photopolymerization are the need for an external light source, possible reaction with embedded compounds and the potential harmful effects of UV light on stability of embedded cells and proteins. Although several groups have shown the compatibility with e.g. fibroblasts [284], radish peroxidase [285] and stem cells [286], the use of UV-light in the presence of biomolecules, cells or tissues remains a controversial topic.

5.3. Double-network hydrogels

Since the mechanical properties of hydrogels formed from solely physical interactions are usually too weak, this especially hampers their applications where mechanical stability is a requirement, such as in load-bearing tissues [287]. On the other hand, *in situ* chemically crosslinkable hydrogels have generally slow gelation kinetics, possibly resulting in early dissolution of the hydrogel. The first hydrogels responding to more than one trigger were prepared by combining pH and temperature induced gelation. Hoffman et al. reported in 1992 about the design of such a hydrogel for the release of amylase [288]. Hydrogels responding to both pH and temperature are generally prepared from copolymers with pH responsive moieties such as *N*,*N*′-diethylaminopropyl methacrylamide or acrylic acid and temperature responsive moieties such as NIPAAm. These hydrogels have been studied e.g. for delivery of insulin [289,290], coenzyme A [291] and indomethacin [292]. Hydrogels can also be built from combined ionically and covalently crosslinked networks to yield so-called interpenetrating networks, which show tremendous synergistic effects on different properties [293,294]. Importantly, combining physical and chemical crosslinking allows the administration of polymers as liquid formulations that undergo quick gelation due to physical interactions triggered by e.g. temperature and that are subsequently stabilized by chemical reactions. As discussed earlier, the most common post-processing reactions are based on reactions between complementary functional groups or covalent crosslinking by radical polymerization [14]. In combination with thermosensitive polymers, dual gelling hydrogels have for example been designed from thiol and vinyl functionalized pNIPAAm [295] and a combination of hyaluronic acid and Pluronic [296]. The gelation kinetics and the resulting properties of the hydrogel are mostly affected by the type of functional groups and the stoichiometry of the two components [251]. P(NIPAAm-co-HEMA) macromers with either alkyne or azide functionalities were *in situ* stabilized by click chemistry [297]. Dual physically and chemically gelling hydrogels have also been formed from thermoresponsive pNIPAAm macromers with epoxy functionalities *in situ* crosslinked with polyamidoamines (PAMAM) [298]. As is shown in Fig. 15, the introduction of this chemical crosslinker significantly increased the swelling of these hydrogels.

Furthermore, swelling behavior and compressive mechanical properties of these hydrogels could be tuned depending on the PAMAM crosslinker length, amine/epoxy ratio, preparation time and polymer concentration [299]. Copolymerization with the hydrolysable monomer dimethyl-γ-butyrolactone acrylate (DBA) resulted in the formation of a bioresorbable hydrogel [300].

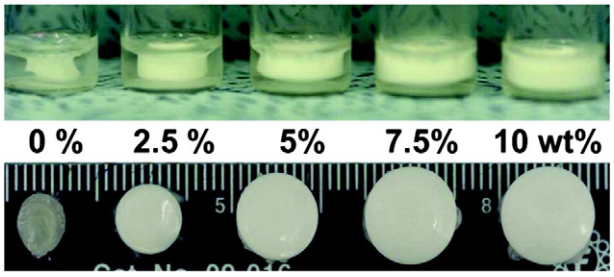

Fig. 15. Swelling of thermoresponsive, chemically crosslinked hydrogels. Increasing the PAMAM crosslinker content resulted in an increased hydrogel swelling.
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In a similar approach, temperature sensitive polymers were combined with supramolecular inclusion complexation between a star-shaped adamantyl-terminated 8-arm PEG and a star-shaped pNIPAAm with a beta-cyclodextrin core (Fig. 16) [301].

Vermonden et al. reported about the design of a thermoresponsive hydrogel that was initially stabilized by physical crosslinks and further crosslinked after UV irradiation [302]. Release of lysozyme, BSA and IgG from these hydrogels was diffusion-controlled and depended on protein size and hydrogel molecular weight between the crosslinks [303]. The secondary structure and enzymatic activity of lysozyme were not affected during gelation. Interestingly, these hydrogels could also be deposited in defined three-dimensional structures using bioprinting technologies [304]. Additive manufacturing techniques offer the possibility to have high control over the scaffold architecture and the fabrication of multi-layered scaffolds. However, hydrogel-inks for 3D bioprinting need to fulfill many requirements with respect to processing parameters, which was recently reviewed by Malda et al. [305].

5.4. Combination of natural and synthetic polymers

In order to obtain materials that are both mechanically strong and bioactive, natural and synthetic polymers have been combined [306,307]. Hydrogels based on natural polymers include collagen, chitosan, fibrin, matrigel, agarose, alginate, hyaluronic acid, cellulose and gelatin [6,308]. Initially, non-chemically crosslinked blends of these polymers were formed, showing enhanced cell adhesion and high tunability over mechanical properties [309–311]. More recently, covalently crosslinked hybrid hydrogels consisting of natural and synthetic polymers have been designed by introduction of functional groups in both polymers. Censi et al. showed the *in situ* stabilization of thiolated hyaluronic acid and (meth)acrylated thermosensitive polymers by Michael addition reaction [312]. Further, these hydrogels facilitated the diffusion-controlled release of bradykinin. PNIPAAm based polymers with a hydrazide functionality have been *in situ* crosslinked with natural carbohydrate polymers such as hyaluronic acid, carboxymethyl cellulose, dextran and methylcellulose, resulting in the formation of a hydrazone bond [313]. Other examples of natural-synthetic hydrogels include combinations of gelatin methacrylamide and PEG [314], fibrin and polyurethane [315] and PEG crosslinked with chitosan [316].

5.5. Composite hydrogels

Incorporation of small inorganic molecules such as calcium phosphate and hydroxyapatite in hydrogels can both enhance the mechanical properties of the hydrogel and promote bioactivity e.g. mineralization for bone tissue engineering applications [317–319]. Ceramics-hydrogel composite materials have been studied for drug delivery in tissue engineering, having controlled scaffold porosity and therefore a tailored drug release [320].

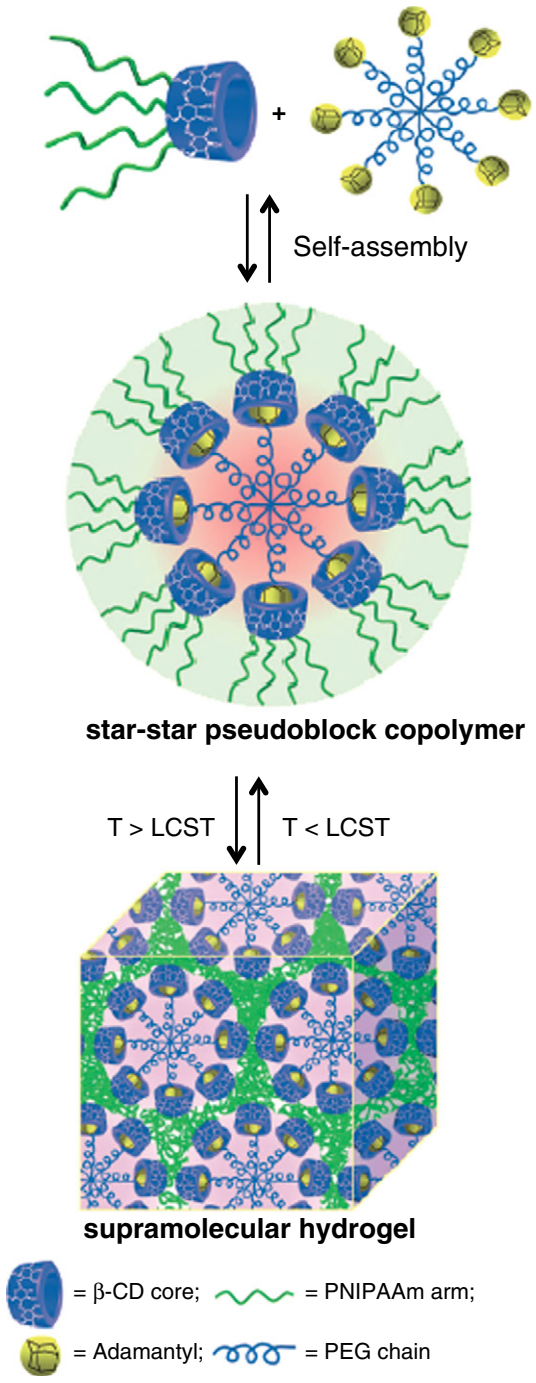

Fig. 16. Combined inclusion complexation and thermogelation in aqueous solution of a star-star shaped copolymer.
Adapted from [301], copyright (2013), with permission from John Wiley & Sons, Inc.

Hybrid and mechanically strong hydrogel constructs can also be formed by alternate deposition of thermoplastic polymer fibers and hydrogels [321]. A hydrogel-electronspun composite showed significantly decreased burst release of BSA from 20 to 7% due to the incorporation of hydrophobic poly(ε-caprolactone)-based fiber mats [322]. Recently, an alginate hydrogel was reinforced with a 3D Ormocomp framework to protect embedded cells [323]. This hybrid hydrogel construct could increase the cell viability and successfully released dopamine.

Additionally, carbon nanotubes have been suggested to increase the mechanical properties of hydrogels and serve as a structural nanofiber. Single-walled carbon nanotubes were mixed with cyclodextrins to form a reversible gel through π–π interactions [324]. Carbon nanotubes combined with naturally-derived polymers such as gelatin increased the stability of the hydrogel at 37 °C without covalent crosslinking [325]. Incorporation of only 2 wt.% carbon nanotubes in hyaluronic acid hydrogels and subsequent crosslinking by divinyl sulfone resulted in a 3-fold higher dynamic modulus [326]. Additionally, incorporating pristine multi-walled carbon nanotubes in a polymethacrylic acid hydrogel allowed the triggered release of ^{14}C-sucrose after electrical stimulations [327].

Incorporating degradable micro- or nanoparticles loaded with a drug in a hydrogel can further extend the possibilities for drug delivery. The drug can be released over a longer time frame, since it first has to leave the particle and subsequently has to diffuse out of the hydrogel matrix [328]. Additionally, these systems can enhance the solubility of hydrophobic drugs in the hydrogel matrix [329]. Finally, microparticles can shield the encapsulated content during hydrogel formation. For example, prednisone acetate has been loaded in poly(N-isopropylacrylamide)-*block*-poly(methyl methacrylate) (pNIPAAm-*b*-PMMA) micelles incorporated in a pNIPAAm hydrogel, resulting in a sustained release [330]. In a different hydrogel-microsphere composite, dexamethasone (DX) and vascular epithelial growth factor (VEGF) were loaded directly either in the hydrogel or in microspheres that were encapsulated in this hydrogel, showing a significant effect on the release kinetics [331]. When the drug was encapsulated in the microspheres, a prolonged release was achieved (Fig. 17).

Composite hydrogels of oligo(poly(ethylene glycol)fumarate) with embedded gelatin microparticles have been developed by the groups of Tabata and Mikos [332]. When these microparticles were loaded with transforming growth factor-β1 (TGF-β1), release kinetics could

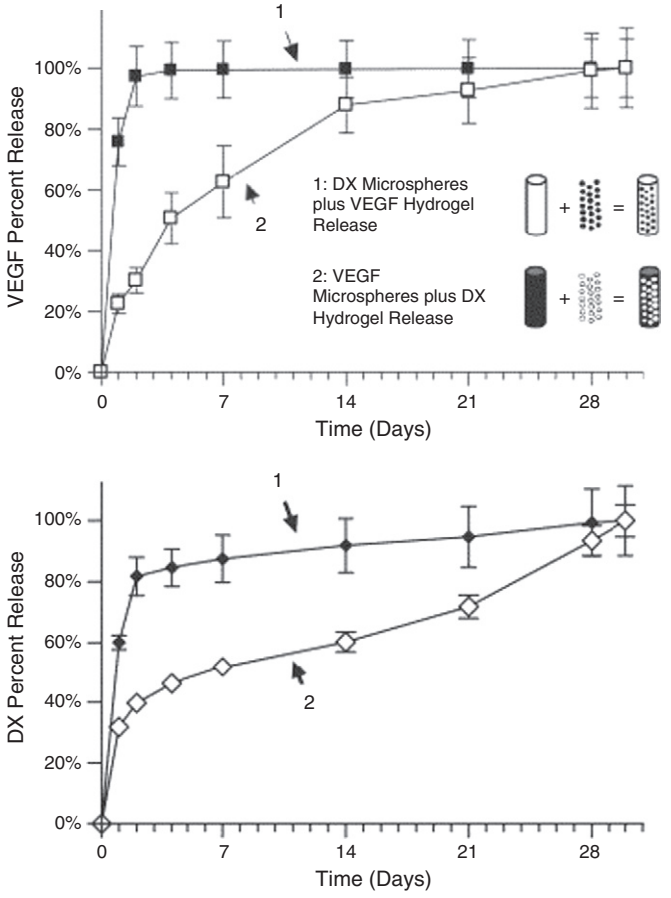

Fig. 17. Release from microsphere/hydrogel composites prepared with loading DX or VEGF either directly in the hydrogel or embedded in the microspheres.
Adapted from [331], copyright (2005), with permission from Elsevier.

be tailored from 13 to 170 pg TGF-β1/day for days 1–3 and from 7 to 47 pg TGF-β1/day for days 6–21 [333]. In combination with encapsulated mesenchymal stem cells enhanced glycosaminoglycan production and an upregulation of cartilage-relevant genes were achieved *in vivo*, showing the potential of these systems for cartilage regeneration [334]. Additionally, these systems have been used for the simultaneous delivery of insulin-like growth factor-1 (IGF-1) and TGF-β1, encapsulated either in gelatin microparticles or directly in the OPF hydrogel, resulting in a high control over release kinetics [335].

6. Conclusions and perspectives

This review describes the progress that has been made in the field of hydrogels for biomedical applications in the past 50 years, starting from the pioneering work of Wichterle and Lim in the 1960s. This historical overview underlines the tremendous development of hydrogels from simple chemically or physically crosslinked networks to complex double network composites. Although not covered in this review, the sophisticated level of these new materials is further reflected in new developments in e.g. shape memory and self-healing hydrogels.

Driven by the need for easy administration and patient convenience an increase in injectable hydrogel formulations has been reported. Stimuli-responsive materials, especially those that quickly respond under mild conditions, form an attractive approach for minimally invasive treatments. The immediate change from a low viscous solution before injection and quick formation of a strong network *in situ* requires careful selection of one or more appropriate crosslinkers. Possibilities to modulate release and degradation profiles after hydrogel administration can further improve the clinical translation of these hydrogels.

We further expect that an increasing knowledge in hybrid or composite hydrogel materials will allow controlled release of more sensitive drugs and several drugs from the same hydrogel matrix. Shielding biopharmaceutical drugs in micro- or nanoparticles can help to maintain their distinct three-dimensional structure. Synergistic hybrid materials fulfill many requirements that cannot be achieved with only one type of polymer. However, an increase in material complexity can also limit the chance of commercialization. Although many polymers have been investigated with high potential, an off-the-shelf hydrogel with highly tunable properties that can provide a platform for multiple applications and patient-specific treatment would be the most promising. Realizing the clinical requirements while limiting the complexity of the hydrogel formulation will therefore be the main goal for the coming decades.

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