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Review

In situ gelling hydrogels for pharmaceutical and biomedical applications

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Abstract

Since Wichterle et al. introduced hydrogels as novel materials possibly suitable for a variety of biomedical applications, hydrogel research has become a fast-developing and exciting research field.

The soft and hydrophilic nature of hydrogels makes them particularly suitable as protein delivery system or as cell-entrapping scaffold in tissue engineering. Traditional hydrogels were formed by chemical crosslinking of water-soluble polymers or by polymerization (of mixtures) of water-soluble monomers. Because of incompatibility of these crosslinking methods with fragile molecules like pharmaceutical proteins and living cells, in recent years research interest has been focused on hydrogels that gel spontaneously under physiological conditions. In these systems, hydrogel formation occurs *in situ*, at the site of injection, without the aid of potentially toxic or denaturizing crosslinking agents. This review provides an overview of *in situ* gelling systems and their potential in biomedical applications. Both photopolymerizable as well as self-assembling hydrogels, based on either chemical crosslinks or physical interactions will be addressed.

Keywords: Hydrogels; *In situ* gelation; Self-assembly; Protein delivery; Tissue engineering

Contents

1. Introduction

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (Peppas et al., 2000). Since the introduction of hydrogels as soft contact lenses in the 1960s (Wichterle and Lim, 1960), their use has increased tremendously and nowadays they are favored in a broad range of pharmaceutical and biomedical applications (Peppas, 1997; Hoffman, 2002; Fedorovich et al., 2007; Van Tomme and Hennink, 2007). Both natural and synthetic polymers can be used for the production of hydrogels. Crosslinking of the polymer chains can be achieved by various

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chemical or physical crosslinking methods (Hennink and van Nostrum, 2002).

The past 5–10 years, research interest has shifted from hydrogel implants to injectable formulations that form a macroscopic gel at the site of injection (Hatefi and Amsden, 2002; Packhaeuser et al., 2004; Kretlow et al., 2007). Several advantages include patient comfort and cost reduction. *In situ* gelation can be obtained after UV-polymerization, introducing non-reversible covalent bonds, or via self-assembly by either reversible interactions or non-reversible chemical reactions. Among these *in situ* gelling systems, self-assembling hydrogels which can be formed in time or in response to a certain stimulus (*e.g.* temperature) and those hydrogels that release their content in reaction to a biological stimulus (*e.g.* glucose concentration (Tanna et al., 2006; Kashyap et al., 2007)), are of most interest. Both physical interactions, *e.g.* electrostatic or hydrophobic interactions, as well as end-group-specific chemical reactions, *e.g.* Michael addition, can be exploited for the design of self-assembly of polymeric networks. In the next sections, various strategies to create *in situ* gelling systems are outlined. Particular interest is given to those systems that are applicable in the pharmaceutical and biomedical field.

2. *In situ* gelling systems

In situ gelling hydrogels can be subdivided into two main categories: systems that are created upon irradiation with visible or UV-light and systems that self-assemble. Photopolymerizable hydrogels are formed *in situ* but are not self-gelling. Self-assembling hydrogels are formed spontaneously or after certain (biological) triggers such as temperature. Table 1 gives an overview of the various *in situ* gelling systems that are discussed in this review. The crosslinking approaches and the specific *in vitro*/*in vivo* evaluations carried out, are included.

2.1. Photopolymerizable hydrogels

In situ photopolymerization has been used in biomedical applications for over more than a decade. The group of Hubbell introduced photopolymerizable hydrogels that could be formed *in situ*. Poly(ethylene glycol) (PEG) was used as central block, which was flanked with oligo(α-hydroxy acids) and acrylate groups, which were coupled to the terminal hydroxyl groups (Sawhney et al., 1993) (Fig. 1A). The acrylate end-groups showed rapid polymerization upon irradiation with visible light in the presence of a suitable photoinitiator to yield a hydrogel structure. The incorporation of the oligo(α-hydroxy acids) oligomers guaranteed the degradability of the matrices under physiological conditions. It was shown that the degradation time of the hydrogels could be tailored from 1 day to 4 months by varying the type of (α-hydroxy acids), *e.g.* glycolic or lactic acid. A continuous release of bovine serum albumin (BSA) from the matrices up to 2 months was achieved, making this system potentially suitable for protein delivery applications. Polymerization of the polymer solution prior to injection, led to the formation of highly hydrated non-adhesive gels. However, when polymerization took place in direct contact with cells, an adherent hydrogel film was formed, presumably as a result of the formation of an interpenetrating network with extracellular proteins, present in tissues. These *in situ* polymerized gels showed to be effective in the prevention of scar adhesion formation after pelvic surgery in animal models (Sawhney et al., 1994). Additionally, it was shown that these hydrogels prevented thrombosis and reduced long-term intimal thickening when applied as a mechanical barrier on severely injured arteries (Hill-West et al., 1994). In a subsequent paper by West et al. the release of proteins and oligonucleotides from the PEG-based hydrogels was investigated in more depth (West and Hubbell, 1995). A linear relationship was found between the molecular weight of the entrapped substance and the release rate. Further, the release was influenced by the molecular weight of the PEG and the type of α-hydroxy acid. It was postulated that the combination of tailorable drug release and *in situ* formation and adherence of the hydrogel to the tissues may allow localized drug release, precisely where needed.

Smeds and colleagues reported on the use of two methacrylate modified polysaccharides, alginate and hyaluronan, that, upon photopolymerization, formed viscoelastic gels (Smeds and Grinstaff, 2001). Alginate, a block copolymer of β-D-mannuronic and α-L-guluronic acid, gels in the presence of divalent cations (*e.g.* Ca^{2+}) (see Section 2.2.3). Hyaluronan (HA), a linear copolymer of 2-acetamide-2-deoxy-D-glucose and D-glucoronic acid, is used clinically in its unmodified form in arthritis treatment and also used as building block for hydrogels. Both polymers were successfully methacrylated and, upon photolysis, yielded viscoelastic gels, possessing mechanical properties similar to those of, *e.g.* nucleus pulposus and meniscus. The HA-based hydrogels were applied *in vivo* after corneal perforation in New Zealand Rabbit eyes. The corneal wound was fully sealed and no vitreous fluid leaked from the eye after three 10 s exposures of a 2% (w/v) polymer solution to an argon ion laser. These *in situ* polymerized hydrogels are promising candidates for sutureless closure of wounds and tissue reconstruction in complex shapes and sizes at not easily accessible sites.

Similarly, Leach et al. reported on photocrosslinkable HA-poly(ethylene glycol) (PEG) polymers for the development of scaffolds (Leach and Schmidt, 2005). HA was modified with glycidyl methacrylate to allow crosslinking. To obtain a denser network, methacrylated HA was copolymerized with acrylated 4-arm PEG (Fig. 1B). The hydrogels were evaluated on their protein releasing potential and showed a tailorable release profile from a few hours to several weeks by altering the crosslink density of the gel or by incorporating poly(lactic-*co*-glycolic acid) (PLGA) microspheres in the hydrogel. Further research is needed to assess the applicability of these systems as *in situ* polymerized tissue engineering scaffolds. Especially the degradability and biocompatibility of the hydrogels are important issues that need to be addressed.

Anseth and co-workers studied PEG and poly(vinyl alcohol)-based polymers, containing acrylate or methacrylate functionalities for the *in situ* generation of photopolymerized networks (Anseth et al., 2002). Their potential in cartilage tissue engineering was illustrated by using a cell-hydrogel construct

Table 1
Overview of polymer systems, crosslinking approaches and type of *in vitro*/*in vivo* evaluations carried out

Polymer	Crosslinking	*In vitro* studies	*In vivo* studies	Reference
PEG-α-hydroxy esters-acrylate	Photopolymerization	BSA release	Prevention of scar adhesion after peritoneal surgery in rats	Sawhney et al. (1993)
		Oligonucleotide release		West and Hubbell (1995)
Methacrylated Alginate Hyaluronan (HA)	Photopolymerization		Wound dressing after corneal perforation in rabbits	Smeds and Grinstaff (2001)
Methacrylated PEG-HA	Photopolymerization	BSA release		Leach and Schmidt (2005)
(Meth)acrylated PEG-PVA	Photopolymerization	Cyto-compatibility		Anseth et al. (2002)
Dextran (dex)-tyramine	H_2O_2 + horseradish peroxidase			Jin et al. (2007)
Thiol-PEG + PEG-divinylsulfone	Reaction between thiol and vinylsulfone	BSA release	Biocompatibility in rats and rabbits	Qiu et al. (2003)
Thiol-heparin + PEG-diacrylate	Reaction between thiol and acrylate	Cyto-compatibility		Tae et al. (2007)
PEG-thioacetate	Oxidation of thiol groups after deprotection + Fe(II)-citrate/lactate + cysteine			Goessl et al. (2004)
Thiol-HA + PEG-diacrylates	Reaction between thiol and acrylate	Cyto-compatibility		Zheng Shu et al. (2004)
Oxidized dex + adipic acid dihydrazide	Reaction between adehyde (AL) and hydrazide			
Oxidized dex + gelatin	Reaction between AL and amino groups			Schacht et al. (1997)
		EGF release		Draye et al. (1998a)
			Biocompatibility	Draye et al. (1998b)
dex-vinylsulfone + 4-arm mercapto-PEG + dex-thiol	Michael-type addition			Hiemstra et al. (2007a,b)
Carboxymethyl dex-hydrazide (HY) + dex-AL or carboxymethyl cellulose-AL	Reaction between HY and AL	Cyto-compatibility	Peritoneal adhesion prevention in rabbits	Ito et al. (2007)
Oxidixed alginate + gelatin	Reaction with proteins in the presence of borax	Cyto-compatibility		Balakrishnan and Jayakrishnan (2005)
HY-PVA + AL-PVA	Reaction between HY and AL	Cyto-compatibility		Ossipov et al. (2007)
Alginate	Complexation with polyvalent cations in lacrimal fluid		Pilocarpine release in rabbit eyes	Cohen et al. (1997)
	Complexation with Ca^{2+}	Cyto-compatibility		Kuo and Ma (2001)
Chitosan	Various chemical and physical crosslinking strategies			Berger et al. (2004a,b)
dex-HEMA-MAA + dex-HEMA-DMAEMA	Ionic interactions between oppositely charged microspheres	Lysozyme, BSA and IgG release		Van Tomme et al. (2005a,b, 2006)
PLGA-PEG-PLGA	Hydrophobic interactions	Release of PEGylated camptothecin		Yu et al. (2008)
Poly(amino acid)-based and hybrid hydrogels	Hydrophobic coiled-coil interactions			Kopecek (2007)
Genetically engineered protein block copolymers	Physical association between coiled-coils			Xu et al. (2005)
dex-PEG, dex-PPG or dex-poly(lysine) + CDs	Inclusion complexes			Huh et al. (2001), Choi et al. (2002, 2005)
dex-L-lactate + dex-D-lactate	Stereocomplexation	Lysozyme and IgG release		De Jong (2001)
		rhIL-2 release	rhIL-2 release in SL-2 lymphosarcoma-bearing mice	Bos et al. (2004)
			Biocompatibility in rats	Bos et al. (2005)
pHEMA-L-lactate + pHEMA-D-lactate	Stereocomplexation			Lim et al. (2000)
PLLA-PEG-PDLA triblock copolymers	Stereocomplexation			Fujiwara et al. (2001)
PEG-PLLA/PDLA diblock- and triblock-copolymers	Stereocomplexation			Li and Vert (2003)
PEG-$(PLLA)_8$ + PEG-$(PDLA)_8$	Stereocomplexation	Lysozyme and IgG release		Hiemstra et al. (2006b)
			rhIL-2 release in SL-2 lymphosarcoma-bearing mice	Hiemstra et al. (2007c)
Methacrylated PEG-$(PLLA/PDLA)_8$	Photopolymerization + stereocomplexation			Hiemstra et al. (2007d)
dex-HEMA-L-lactate + dex-HEMA-D-lactate microspheres	Hydrophobic interactions and stereocomplexation	Lysozyme release		Van Tomme et al. (2008)

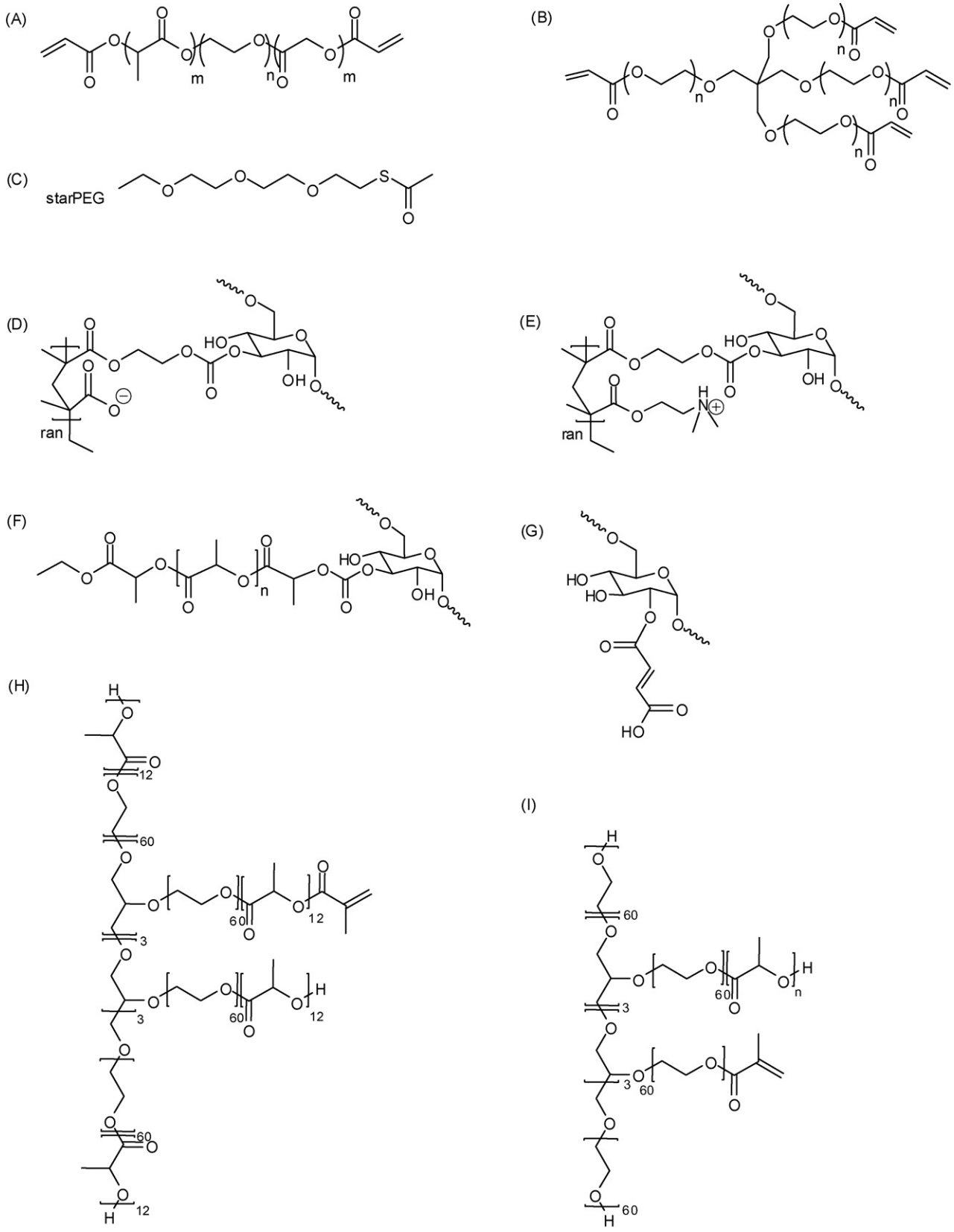

Fig. 1. (A) PEG flanked with oligo(α-hydroxy acids) and acrylate groups, (B) acrylated 4-arm PEG, (C) star-shaped PEG-thioacetate, (D) dex-HEMA-MAA, (E) dex-HEMA-DMAEMA, (F) dex-lactate, (G) dex-MA (dextran-maleic acid), (H) eight-arm PEG-PLA_{12}-MA star block copolymers, (I) PEG-MA/PLA_n ($n = 12$ or 16) star block copolymers.

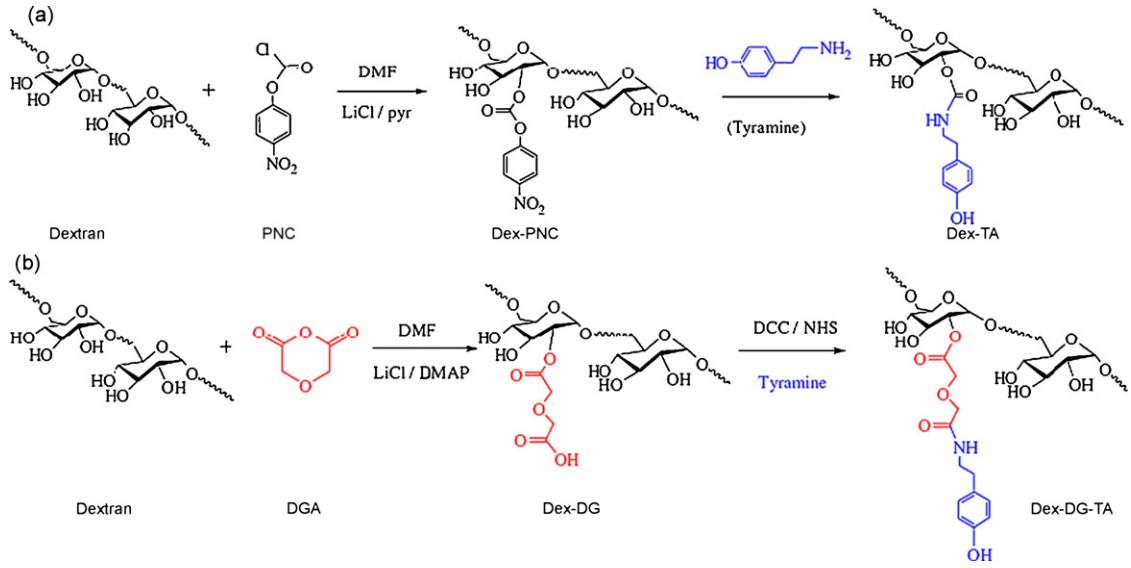

Fig. 2. Preparation of dextran-tyramine conjugates: (a) dex-TA and (b) dex-DG-TA (reprinted from Jin et al. (2007), with permission from Elsevier).

that was rapidly formed after photopolymerization and temporarily served as a replacement for the damaged cartilage while new cartilage was formed.

A review by Nguyen and West summarizes the potential applications of photopolymerizable hydrogels in tissue engineering (Nguyen and West, 2002). Additionally, the advantages of photopolymerization and the available polymerizable materials and photoinitiators are outlined.

2.2. Self-assembling hydrogels

2.2.1. Enzyme-mediated gelation

Dextran-tyramine (dex-TA) conjugates were *in situ* crosslinked in the presence of H_2O_2 and horseradish peroxidase (HRP), yielding chemically crosslinked, highly elastic and degradable hydrogels (Jin et al., 2007) (Fig. 2). The HRP-mediated coupling reaction of phenol moieties in dex-TA conjugates occurred via a carbon–carbon bond at the ortho positions and/or via a carbon–oxygen bond between the carbon atom at the ortho position and the phenoxy oxygen (Fig. 3). The gelation time could be varied from 5 s to 9 min, depending on the polymer concentrations and enzyme or H_2O_2/tyramine ratios. Degradation of the gels (denoted as dex-TA) took several months due to the slow hydrolysis of the urethane bonds. In related hydrogels where the tyramine units were linked to the dextran via an ester-containing diglycolic group (denoted as dex-DG-TA), degradation occurred more rapidly within 4–10 days. For rheological analysis, Jin et al. made use of a double syringe equipped with a mixing chamber. One compartment contained a solution of the dextran-tyramine conjugate, whereas the other one contained a mixture of H_2O_2 and HRP. *In vivo* relevance of this novel system needs to be investigated, but it can be postulated that the use of a dual syringe will facilitate the administration of the hydrogel system.

2.2.2. Chemical crosslinking of complementary groups

PEG has been frequently used for the preparation of hydrogels, mainly because of its proven safety and FDA approval of PEG-based systems for internal use (Bailey and Koleske, 1976). Modification of PEG with photopolymerizable (meth)acrylate groups has been discussed in the previous section, whereas the use of PEG in thermosensitive triblock copolymer gels will

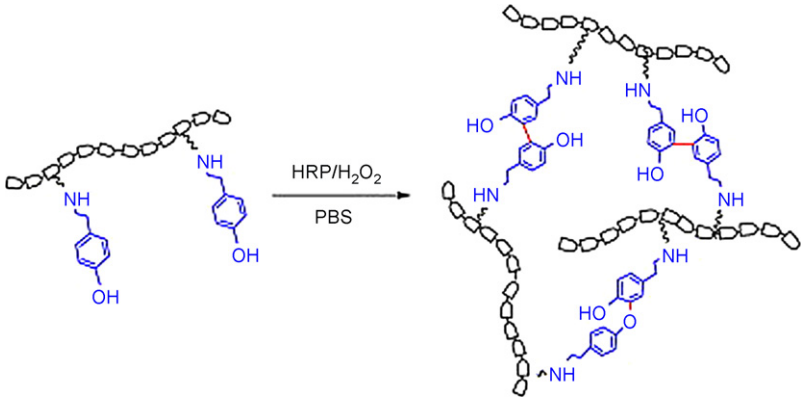

Fig. 3. Enzymatic crosslinking of dextran-tyramine conjugates (reprinted from Jin et al. (2007), with permission from Elsevier).

be discussed in Section 2.2.4. Qiu and colleagues reported on the use of PEG, functionalized with thiol groups, for the design of self-assembling, chemically crosslinked hydrogels (Qiu et al., 2003). Diamino-PEG was copolymerized with 2-mercaptosuccinic acid to yield an amide-linked copolymer with pendant thiol groups, which in turn reacted with a PEG-divinylsulfone (Fig. 4). The vinylsulfone groups react quite rapidly under mild conditions (pH 7, RT) with thiol groups resulting in hydrogels with a water content above 90%.

A continuous *in vitro* release of fluorescein-labeled BSA was observed during 25 days with no significant burst release. Quantitative release of the entrapped protein was found, indicative of the mild crosslinking conditions. After subcutaneous injection of the gels in rats and rabbits, minimal inflammatory cell response was observed, with a mild to moderate fibrous capsule formation and modest numbers of macrophages around the material.

The group of Hoffman also made use of the reactivity of thiol groups with acrylates (Tae et al., 2007). In their study, heparin was functionalized with thiol groups and subsequently reacted with PEG-diacrylate to form a hydrogel (Fig. 5). With increasing functionalization of heparin, its bioactivity, characterized by the affinity to antithrombin III, decreased.

It was shown that the mechanical properties (storage modulus) and gelation kinetics could be tailored by varying the degree of thiolation and the total precursor concentration (both heparin-SH and PEG-diacrylate). Keeping the applicability as *in situ* forming gelation system in mind, it was concluded that at least 30% thiolation was required for an appropriate gelation time (∼5 min). Successful encapsulation of cells was observed, indicative of the non-cytotoxicity of the material. Additionally, it was shown that cell proliferation could be enhanced by addition of fibrinogen during gelation.

Uncontrolled dimerization upon synthesis and storage of thiol-functionalized polymers is a frequently encountered issue.

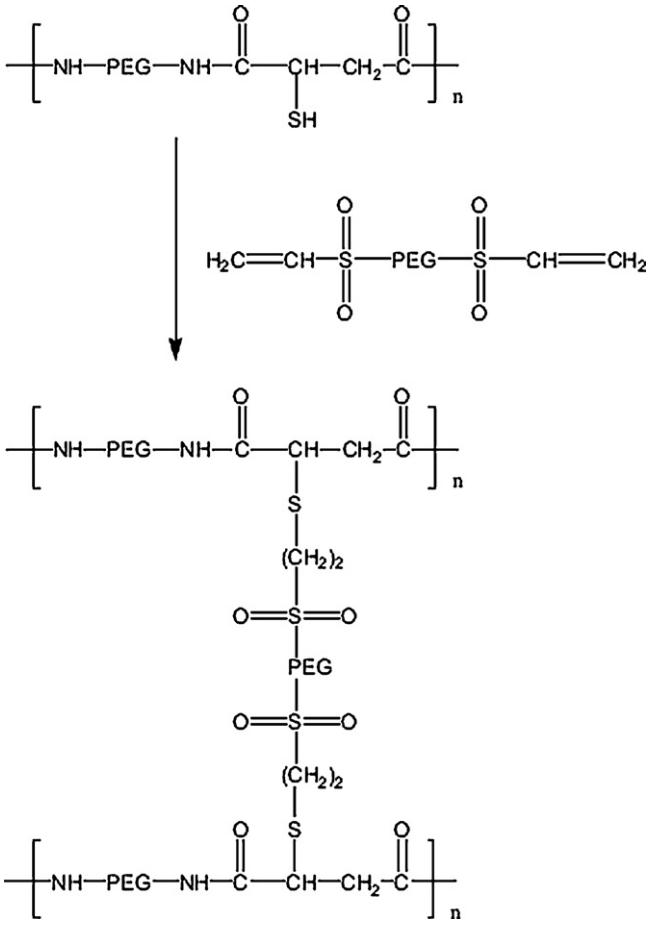

Fig. 4. Hydrogels formed through spontaneous chemical crosslinking of PEG-based copolymers (reprinted from Qiu et al. (2003), with permission from Elsevier).

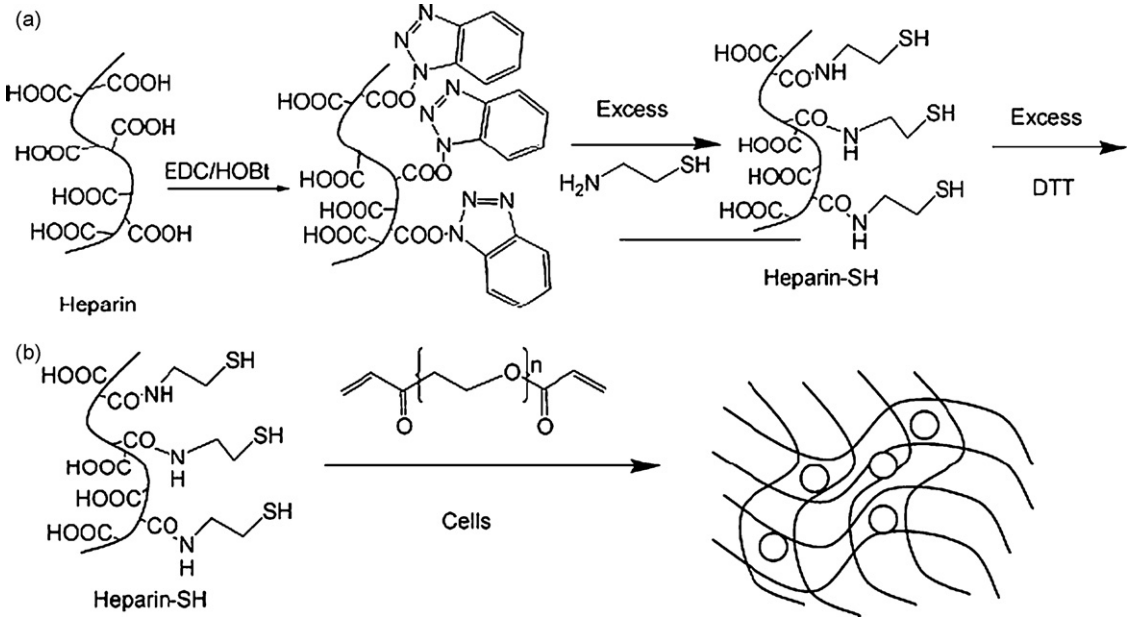

Fig. 5. *In situ* gelation of thiol-functionalized heparin with PEG-diacrylate (reprinted from Tae et al. (2007), with permission from ACS publications).

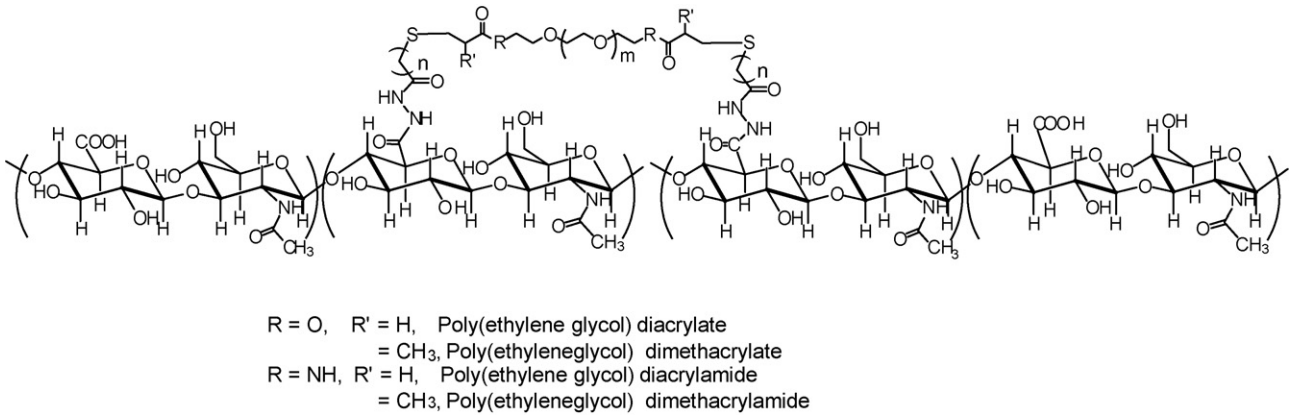

Fig. 6. Structures of α,β-unsaturated esters and amides of PEG crosslinked with thiolated HA (reprinted from Zheng Shu et al. (2004), with permission from Elsevier).

This was circumvented by the group of Hubbell (Goessl et al., 2004) by the use of thioacetate as a stable, protected thiol group. The resulting precursor molecule, star-shaped PEG-thioacetate (Fig. 1C), could be stored at ambient conditions and could be rapidly and quantitatively deprotected using a base (*e.g.* 0.1 M NaOH), yielding free thiol groups and acetate. Subsequently, oxidation of the thiols by molecular oxygen resulted in hydrogel formation. After injection of an oxygen-free aqueous polymer solution into an experimental animal, oxygen diffusion from surrounding tissues led to oxidation of the thiol groups, resulting in the formation of disulphide linkages to yield a hydrogel. However, since gelation was too slow to be clinically useful (more than 1 h), a Fe(II)-citrate/lactate complex in combination with cysteine was used to accelerate the crosslinking reaction and to decrease the gelation time to 90 s. The authors hypothesize that the system could be of interest in drug and gene delivery or as anti-adhesive, protein repellant hydrogel film in, *e.g.* angioplasty or interperitoneal surgery.

Thiol-functionalization was also applied by Zheng Shu and co-workers for the design of HA-based hydrogels for tissue engineering (Zheng Shu et al., 2004). Thiolated HA derivatives were coupled to α,β-unsaturated esters and amides of PEG (Fig. 6). The PEG-diacrylates showed to be most reactive with cysteine and in a molar ratio of 2:1 At physiological conditions, HA:PEG gelation occurred within 9 min, which is likely suitable for an *in situ* crosslinking application. A low *in vitro* cytotoxicity was found after suspending human tracheal scar fibroblasts in the HA solution, prior to addition of the PEG solution.

Besides HA, dextran is an interesting polysaccharide for the preparation of *in situ* crosslinked hydrogels. Maia et al. described the oxidation of dextran with sodium periodate to yield dexOx (Fig. 7). The subsequent self-assembly of this aldehyde-functionalized dextran with hydrazide groups of adipic acid dihydrazide (AAD) resulted in the formation of injectable hydrogels (Maia et al., 2005). Swelling and degradation of the hydrogels was dependent on the number of intermolecular crosslinks which in turn depends on the AAD content. The degradation time at physiological conditions of gels consisting of 15% oxidized dextrans was 9, 15 and 23 days, for gels with, respectively, 5, 10 and 20% of AAD. Additionally, it was found that the pore size increased during degradation of the network from 1.5 to 6.3 μm after 11 days, which might promote the ingrowth of cells and result in a better integration of the matrix with the surrounding tissue.

In situ reaction through Schiff's base formation of aldehyde groups of oxidized dextran with amino residues in gelatin was used by Schacht et al. for the preparation of self-gelling hydrogels (Schacht et al., 1997). The gelation time depended on the pH and ionic strength of the buffer and was typically about 3 min in phosphate buffer of pH 7 (ionic strength not mentioned). The strength of the gels increased with the extent of dextran oxidation. Sustained release of epidermal growth factor (EGF) *in vitro* from hydrogel films was observed up to 7 days (Draye et al., 1998a). *In vivo* biocompatibility studies of gelatin-oxidized dextran composites showed a moderate foreign body reaction around the subcutaneous implants (Draye et al., 1998b).

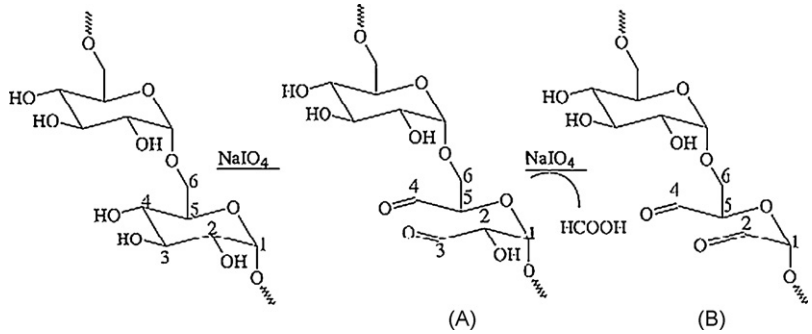

Fig. 7. Oxidation of dextran by sodium periodate: (A) first oxidation and formation of aldehyde groups at C3 and C4, (B) second oxidation with release of formic acid (reprinted from Maia et al. (2005), with permission from Elsevier).

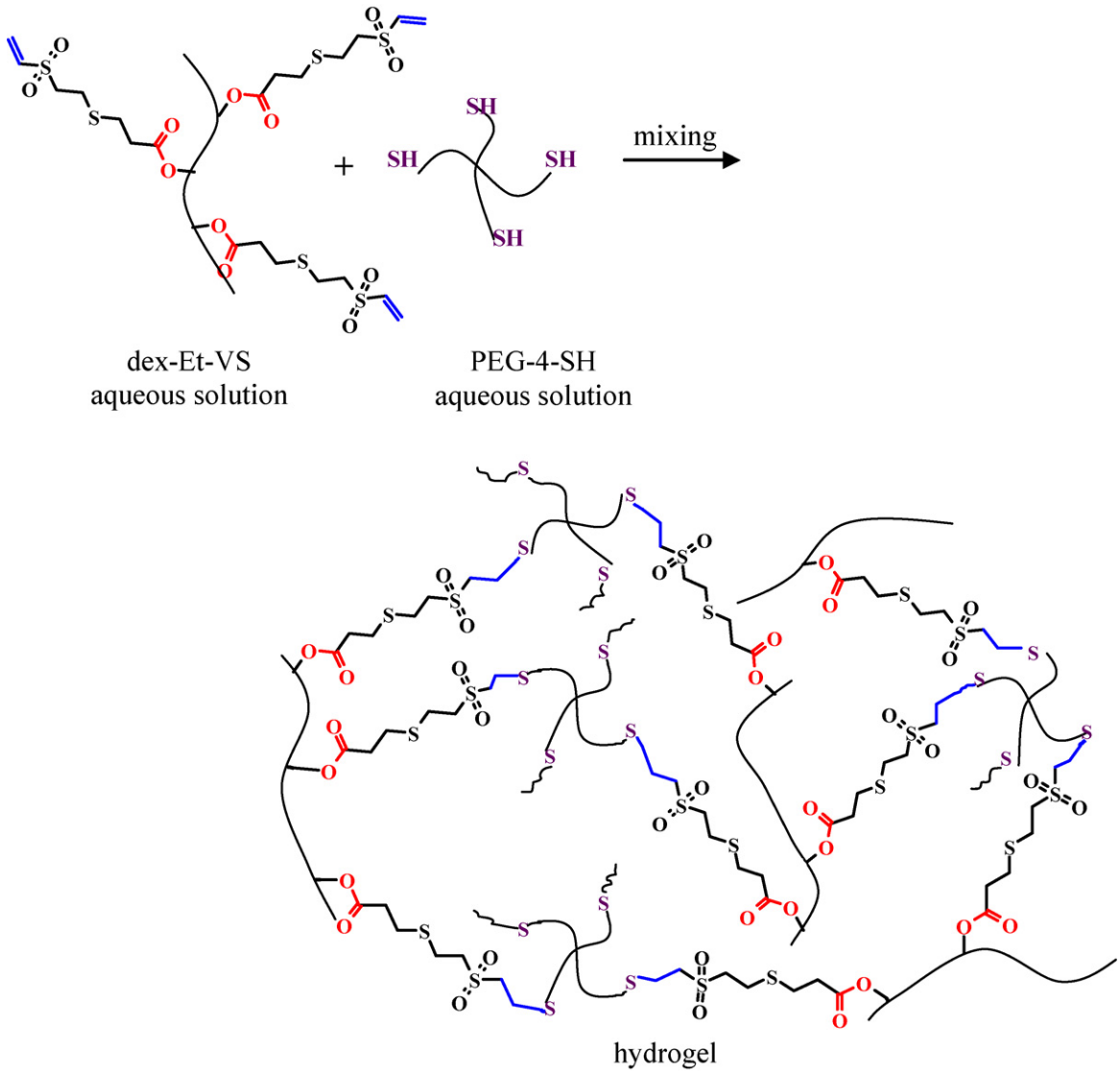

Fig. 8. Schematic presentation of self-assembly of dex-VS and PEG-4-SH via Michael-type addition reaction (reprinted from Hiemstra et al. (2007a), with permission from ACS Publications).

Dextran-based *in situ* gelling systems were also described by the group of Feijen (Hiemstra et al., 2007a). Dextran was functionalized with vinyl sulfone (dex-VS) and subsequently mixed (1:1 molar ratio) with linear of 4-arm mercato-PEG (PEG-4-SH) (Fig. 8). Via a Michael-type addition reaction a hydrogel was formed at physiological conditions. The gelation time decreased from 7 to 0.5 min when the degree of VS substitution (DS, *i.e.* number of VS groups per 100 glucopyranose units) increased from 4 to 13. Rheological analysis showed that highly elastic hydrogels were formed, of which the strength could be tailored from 3 to 46 kPa, by varying the DS, concentration and dextran molecular weight. Additionally, the degradation time of the *in situ* gelling systems could be tailored from 3 to 21 days, by changing the same parameters. In a subsequent paper, dextran was functionalized with thiol (dex-SH) and mixed with either dex-VS or PEG-4-SH resulting in *in situ* hydrogel formation (Hiemstra et al., 2007b). In this way, the degradation times of the gels could be prolonged up to 21 weeks while the storage moduli could be increased to 100 kPa.

Ito et al. described hydrogel formation upon mixing of hydrazide-modified carboxymethyldextran (CMDX-ADH) with aldehyde-modified dextran (DX-CHO) or carboxymethylcellulose (CMC-CHO) (Ito et al., 2007). The crosslinked hydrogels showed mild to moderate *in vitro* cytotoxicity. Because of dextran's proven biocompatibility, the hydrogels were tested on their applicability to prevent peritoneal adhesions, a serious consequence of abdominal surgery that can lead to pain, bowel obstruction or even infertility (diZerega and Campeau, 2001). A double-barreled syringe was used to inject the two hydrogel precursors into female rabbits at the site of peritoneal defects and abrasions. Rabbits treated with CMDX-DX developed adhesions that were larger than the saline control group, whereas with CMDX-CMC hydrogels a clear reduction in adhesion formation was observed. Overall, the authors conclude that their

in situ crosslinked hydrogels are promising systems for the prevention of peritoneal adhesions due to a low cytotoxicity and a slow degradation.

The ability of oxidized alginate to spontaneously crosslink with proteins in the presence of sodium tertraborate (borax) resulting in hydrogels, was explored by Balakrishnan and Jayakrishnan (2005) (also see Sections 2.1 and 2.2.3 for other crosslinking strategies for alginate-based gels). Sodium alginate was oxidized with periodate and subsequently dissolved in borax-containing PBS and mixed with an aqueous solution of gelatin, after which instantaneous gelation occurred (Fig. 9). Injectability was assessed by using a double syringe in which one compartment was filled with gelatin solution and the other with oxidized alginate and borax. *In vitro*, within seconds after extrusion from the needle a hydrogel was formed. No data on the *in vivo* applicability of this system have been reported, yet. Furthermore, it was shown that neither the gels nor their extracts showed any cytotoxicity towards mouse fibroblasts. Additionally, encapsulation of hepatocytes inside the matrix demonstrated the suitability of these hydrogels for tissue engineering applications. It was found that after 2 weeks, the protein producing ability of the cells was preserved and after 4 weeks the hepatocytes still retained their characteristic morphology.

Hydrazone formation between hydrazide (HY) and aldehyde (AL) groups was also used by Ossipov et al. for the *in situ* preparation of poly(vinyl alcohol) (PVA)-based injectable hydrogels (Ossipov et al., 2007) (Fig. 10). Mixing solutions of both components resulted in immediate gelation when the polymer concentration was 5 wt%, whereas for 2.5 wt% the gelation time was 10 min and for 1.25 wt% no gel formation occurred. Higher polymer concentrations could not be used due to the poor solubility of the PVA derivatives. The applicability of the hydrogels in tissue engineering was assessed by encapsulating murine neuroblastoma cells in the hydrogels upon mixing of the two gel components. It was shown that the cells could be homogeneously entrapped in the gels without loss of cell viability. Up to 4 days after encapsulation, the cells were still viable, but after 5 days they died. Apparently, the non-degradability of the matrix does not allow cells to proliferate due to insufficient space. The authors suggest that the incorporation of degradable

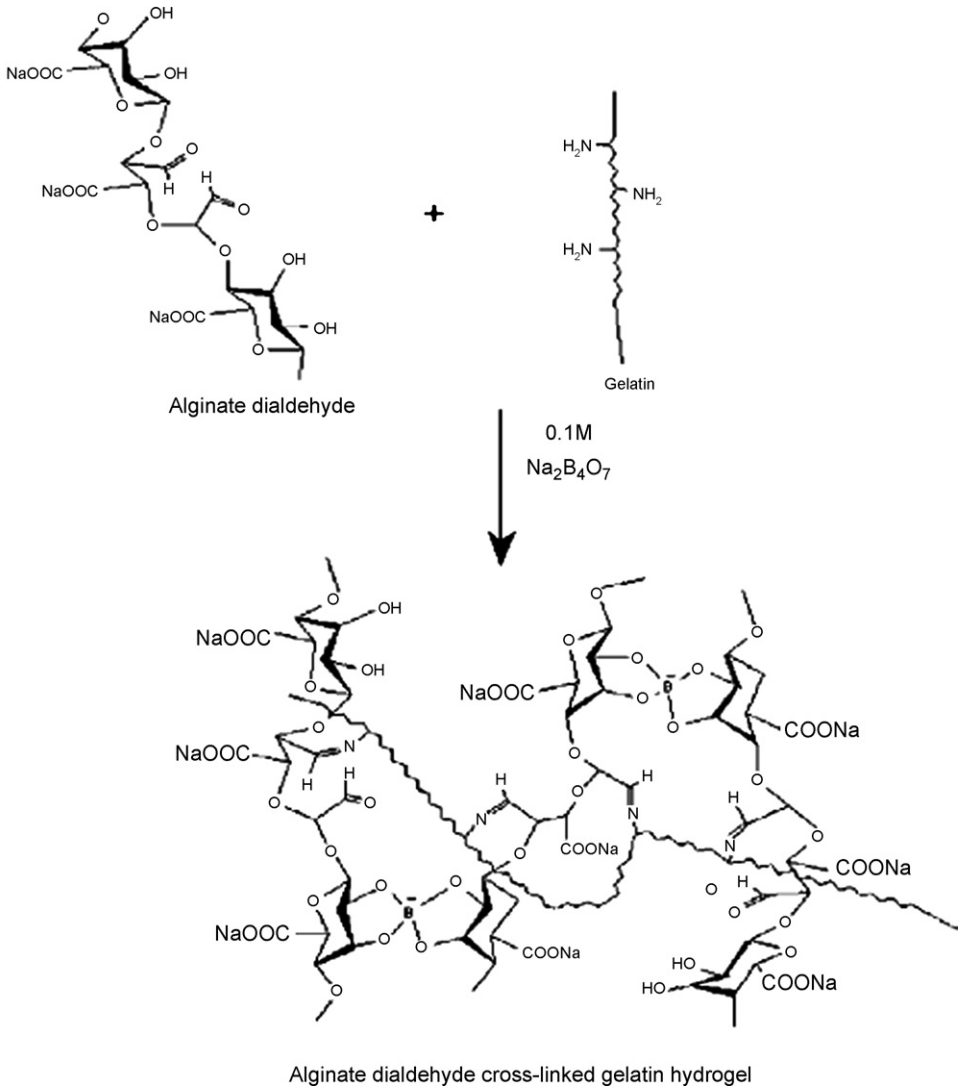

Fig. 9. Oxidized alginate crosslinks with gelatin in the presence of borax (reprinted from Balakrishnan and Jayakrishnan (2005), with permission from Elsevier).

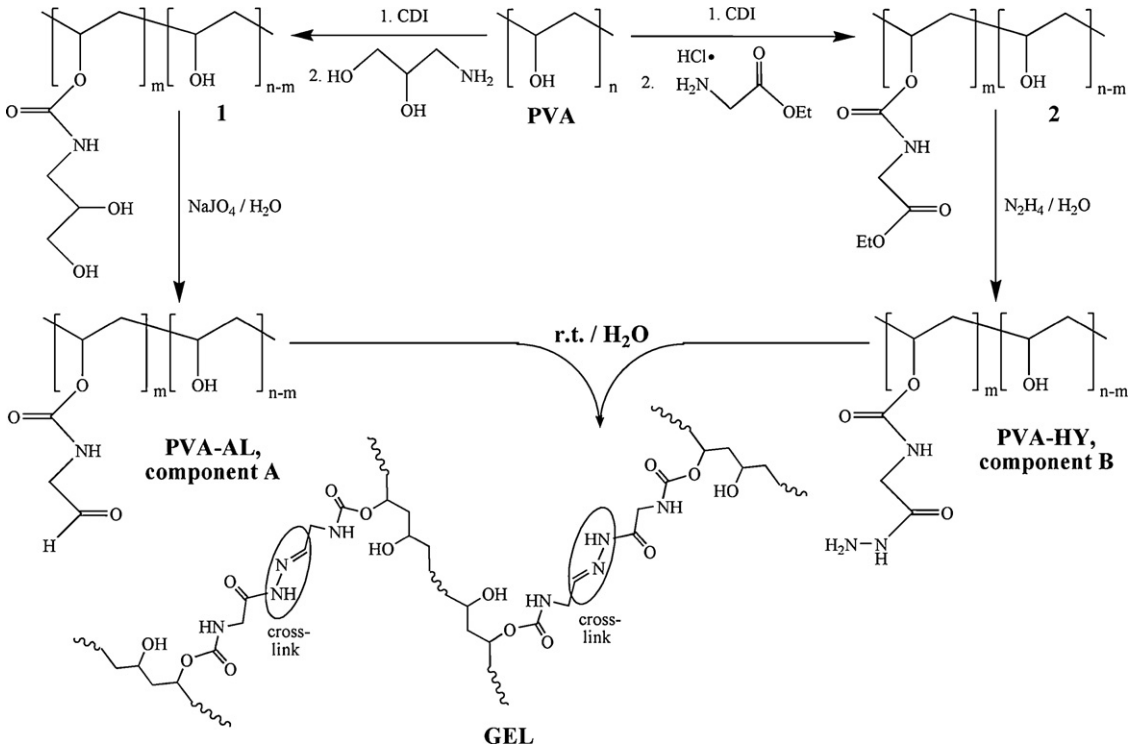

Fig. 10. Synthesis of PVA-AL and PVA-HY and the *in situ* crosslinking leading to hydrogel formation (reprinted from Ossipov et al. (2007), with permission from Wiley Interscience).

linkers between the PVA backbone and the functional groups could be a valuable option to overcome this problem. These results illustrate the importance of biodegradability of hydrogel scaffolds in tissue engineering.

2.2.3. Ionic interactions

As mentioned in Section 2.1 alginate, derived from sea weed, is an anionic linear copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G), arranged as homopolymeric M–M and G–G blocks, together with alternating M–G blocks. By complexation of bi- or poly-valent cations with the G moieties, three-dimensional hydrogels are formed (Grant et al., 1973). The gelation time, mechanical properties and drug release from alginate matrices is dependent on the G:M ratio, the polymer concentration and the type of cation. Calcium crosslinking has shown to yield gels with good mechanical properties.

Cohen et al. reported on the use of alginate as *in situ* forming hydrogel for the controlled release of pilocarpine in the eye (Cohen et al., 1997). It was found that alginates with a G content of more than 65% instantaneously formed hydrogels upon addition of lacrimal fluid, whereas alginates with a low G content relatively slowly formed only weak hydrogels. A diffusion-controlled release of pilocarpine from alginate gels for 24 h was observed. Additionally, a prolonged pharmacodynamic effect was obtained as shown by an intra-ocular pressure reduction for 10 h, when compared to 3 h for pilocarpine nitrate solution.

Alginate hydrogels have also been investigated on their potential in tissue engineering applications (Kuo and Ma, 2001). The influence of the calcium salt on the gelation properties was studied. It was found that, when $CaCl_2$ was used, the gelation rate was hard to control, resulting in inhomogeneous and mechanically weak hydrogels. The use of $CaCO_3$ (with or without $CaSO_4$), on the other hand, led to a slower gelation, allowing the formation of homogeneous scaffolds with defined macroscopic dimensions. Osteoblasts could be uniformly entrapped in the gels and the homogeneity of the pores ensured nutrient diffusion through the scaffold and thus cell growth. These hydrogels could be injected into the body at specific sites and gel *in situ* into complex geometries, dependent on the defect to be filled.

Chitosan (an alternating copolymer of β-2-acetamido-2-deoxy-D-glucopyranose and 2-amido-2-deoxy-D-glucopyranose) is obtained by deacetylation of the natural occurring chitine found in, *e.g.* the skeleton of crustaceans. It is an attractive polymer for biomedical applications due to its biodegradability (Muzzarelli, 1997), bioadhesiveness (He et al., 1998), penetration enhancing effect by opening epithelial tight junctions (Kotzé et al., 1999) and, its ability to condense DNA through electrostatic interactions and, as a result, the potential application in gene delivery (Sato et al., 2001). Moreover chitosan can be used in the formation of hydrogels, both chemically and physically crosslinked. Chemical crosslinking of chitosan hydrogels is most commonly achieved via the formation of covalent imine bonds between dialdehyde crosslinkers, such as glutaraldehyde, and chitosan amino groups (Monteiro and Airoldi, 1999). Physical crosslinking can be achieved by, *e.g.* ionic interactions between the polycationic chitosan and metallic anions (*e.g.* Pt(II) (Brack et al., 1997)) or tripolyphosphate (Mi et al., 1999). Berger and co-workers published two extensive reviews on chitosan hydrogels, the crosslinking strategies

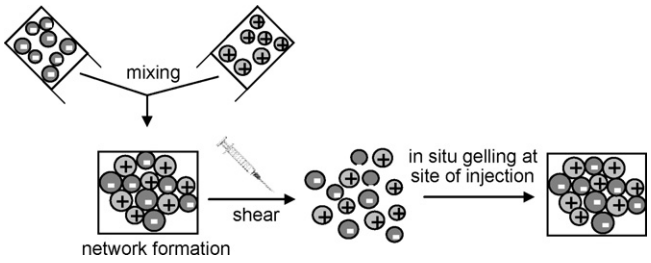

Fig. 11. Schematic presentation of the self-assembling microsphere-based dextran hydrogel (reprinted from Van Tomme et al. (2005b), with permission from Elsevier).

that can be used and the potential applications in the biomedical field (Berger et al., 2004a,b).

Recently, our group reported on the formation of self-assembling dextran hydrogels, based on ionic interactions between oppositely charged dextran microspheres (Van Tomme et al., 2005b). Charged microspheres were prepared by copolymerization of either methacrylic acid (MAA) or *N*,*N*-dimethylamino ethyl methacrylate (DMAEMA) with hydroxyethyl methacrylate-derivatized dextran (dex-HEMA). Rheological analysis confirmed that mainly elastic networks were formed, instantly upon mixing of equal volumes of aqueous dispersions of the anionic dex-HEMA-MAA (Fig. 1D) and cationic dex-HEMA-DMAEMA (Fig. 1E) microspheres at pH 7. The hydrogel strength could be tailored by varying the water content of the gel. Importantly for applications, it was shown that above a certain applied shear stress, the system starts to flow, whereas once the stress is removed, the network rebuilds, making it particularly attractive as injectable matrix (Fig. 11).

In a subsequent study, protein mobility in the hydrogels and protein release from the matrices were investigated (Van Tomme et al., 2005a). The gels could be loaded with proteins by simply mixing the microsphere dispersions with a protein solution, avoiding the use of potentially damaging factors (organic solvents, extreme pH, temperature, etc.). At pH 7, a diffusion-controlled release of the proteins, in accordance to their hydrodynamic radii, was observed with 50% of the initial amount of lysozyme, BSA and IgG released in respectively 4, 6 and 13 days (Fig. 12). Lysozyme was quantitatively released in about 25 days, with full preservation of its enzymatic activity, confirming the protein-friendly preparation technology.

The degradation behavior of the charged microspheres and macroscopic gels was described in a succeeding paper (Van Tomme et al., 2006). By varying the crosslink density of the microspheres and the water content of the gels (75–85%) the degradation times of the gels could be tailored from 65 to 140 days. Furthermore, it was demonstrated that the degradation times increased from 30 to 75, 85, 105 and 125 days for 100/0, 75/25, 50/50, 25/75 and 0/100 positively/negatively charged microspheres, respectively. Incorporation of rhodamine-B-dextran (Mw 70,000 g/mol) in both the anionic and cationic microspheres resulted in a degradation-controlled release with a tailorable release profile depending on the positively/negatively charged microspheres ratio. The authors state that the possibility to tailor the network properties

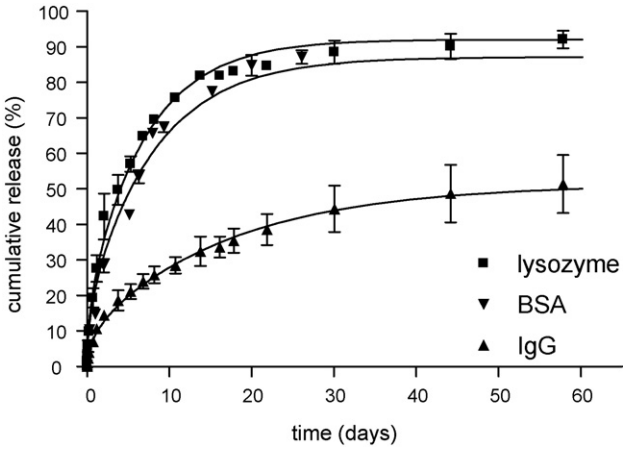

Fig. 12. Cumulative release of lysozyme (■), BSA (▼) and IgG (▲) from hydrogels (15% solid, DS 8) (reprinted from Van Tomme et al. (2005a), with permission from Elsevier).

and degradation behavior of these *in situ* gelling systems makes them suitable candidates for drug delivery and tissue engineering applications.

2.2.4. Hydrophobic interactions

2.2.4.1. Temperature-sensitive hydrogels. By far the most studied type of physical interactions, used for crosslinking of hydrogels, are hydrophobic interactions. Temperature increase and, as a result, dehydration of polymer chains leads to the formation of hydrophobic domains and eventually transition of an aqueous liquid to a hydrogel network. Most studied materials are block copolymers of poly(ethylene oxide) (PEO) and poly(propylene oxide) (PPO), well known as Pluronics®, and PEO-PL(G)A or other polyesters (Jeong et al., 1997, 2000; Zentner et al., 2001; Lee et al., 2006; Jiang et al., 2007). As a typical example, Yu et al. reported on a series of thermogelling PLGA-PEG-PLGA triblock copolymers for the sustained release of PEGylated camptothecin (Yu et al., 2008). Above the critical gelation concentration (CGC), the triblock copolymers exhibited a temperature-dependent reversible sol–gel transition in water (Fig. 13). The sol–gel transition temperature was influenced by the copolymer composition and specifically by the PEG molecular weight and the lactic acid/glycolic acid (LA/GA)ratio of the PLGA blocks. As a result, these parameters also determined the degradation and drug release behavior of the system. Copolymers 1 and 2 (Fig. 13) gelled between room temperature and body temperature and could be used as injectable drug delivery system. A sustained release of the model drug up to 1 month could be obtained, characterized by diffusion of the drug during the first stage, followed by a combination of diffusion and degradation-controlled release.

Other types of temperature-sensitive hydrogels are based on polymers that possess a so-called lower critical solution temperature (LCST) above which they are insoluble and form a hydrogel. The most intensively researched polymers that exhibit this particular behavior are poly(*N*-isopropylacrylamide) (pNiPAAm)-based (Zhang et al., 2003; Huang et al., 2004;

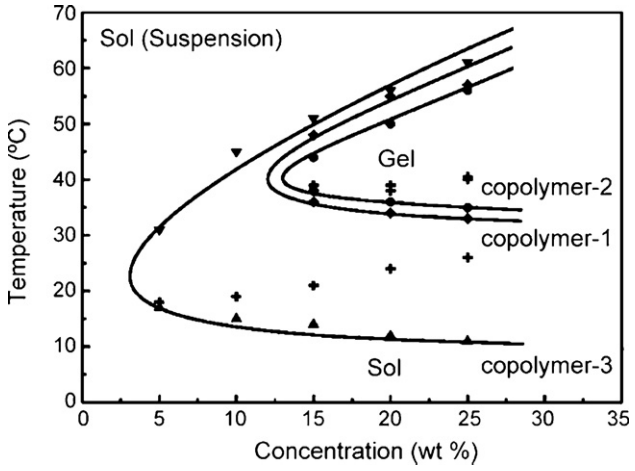

Fig. 13. Phase diagrams of PLGA-PEG-PLGA aqueous solutions. Copolymer 1: PEG 1500 Da, LA/GA 10; Copolymer 2: PEG 1500 Da, LA/GA 5; Copolymer 3: PEG 1000 Da, LA/GA 2.7 (reprinted from Yu et al. (2008), with permission from Elsevier).

Salgado-Rodriguez et al., 2004; Huang and Lowe, 2005) or more recently, poly(2-hydroxypropyl methacrylamide)-based (Vermonden et al., 2006). A major disadvantage of the pNIPAAm-containing hydrogels is the non-biodegradability of the thermosensitive polymer. Therefore, these polymers are frequently combined with polymers such as dextran, to pursue partial biodegradability. However, the *in vivo* fate of these hybrid networks remains unclear and needs thorough investigation.

The hydrogels described above are frequently referred to as 'stimuli-responsive', since they are formed or release their content in response to a physical stimulus, in these particular cases, the temperature. These hydrogel systems have been the topic of extensive reviews the past decade (Bromberg and Ron, 1998; Jeong et al., 2002; Ruel-Gariepy and Leroux, 2004; Klouda and Mikos, 2008). The main advantages of this type of hydrogels are their convenient administration and the tailorability of their physical properties in response to particular physiological stimuli.

2.2.4.2. Peptide-based hydrogels. Nowadays, there is a growing interest in supramolecular peptide-based or hybrid hydrogels (Pochan et al., 2003; Hamley et al., 2005; Xu et al., 2005; Mahler et al., 2006; Veerman et al., 2006; Haines-Butterick et al., 2007). In the latter case, synthetic polymers are combined with protein domains that self-assemble through hydrophobic coiled-coil interactions. The coiled-coil is one of the basic folding mechanisms of native proteins and comprises a superhelix that consists of two or more α-helices winding together (Yu, 2002). These poly(amino acid)-based and hybrid hydrogels have been recently reviewed by Kopecek and colleagues (Kopecek, 2007; Kopecek and Yang, 2007). Xu et al. reported on reversible stimuli-responsive hydrogels composed of genetically engineered protein block copolymers, in which a central water-soluble random coil polyelectrolyte was flanked by 2 coiled-coil domains (Xu et al., 2005). It was found that self-assembly occurred in response to temperature and pH, by physical association between the coiled-coils and dependent on the protein concentration. The thermal stability could be tailored by changing the amino acid sequence in the coiled-coil domains. SEM imaging showed that the hydrogels had a porous interconnected structure. Further research is needed to assess the potential in biomedical applications.

2.2.4.3. Polymer inclusion complexes. The supramolecular assembly of cyclodextrin-modified polymeric host and hydrophobic, low molecular weight guests into injectable polymer inclusion complexes, has been studied by the group of Yui (Choi and Yui, 2006). Cyclodextrins (CDs) are cyclic water-soluble oligosaccharides with internal hydrophobic cavities. It is known that PEG and poly(propylene glycol) (PPG) form inclusion complexes with α-CDs and β-CDs, respectively. PEG and PPG were grafted to a dextran backbone and subsequently mixed with a CD solution. This led to threading of the CDs onto the PEG and PPG chains. Subsequently, gelation occurred, induced by physical crosslinking between the inclusion complexes, *i.e.* hydrogen bonding between the CD clusters along the PEG/PPG chains (Huh et al., 2001; Choi et al., 2002). The time required for gel formation could be tailored by varying the concentration of the graft copolymer and the PEG/PPG content, *i.e.* the graft density on the dextran chains. It was found that above a certain temperature, the supramolecular assembly dissociates yielding a viscous solution, but after cooling down to a specific temperature an opaque gel was reformed. In a subsequent study, in addition to the thermoreversible gelation a pH-sensitive functionality was introduced by grafting the dextran with a cationic polymer, namely poly (ε-lysine) (PL) (Choi et al., 2005). At high pH, the primary amines of PL are deprotonated, allowing the CDs to be threaded onto the PL chain, while at pH 4, a rapid gel-to-sol transition occurred, due to dissociation of the inclusion complexes in the protonated state of PL. Again, the phase transition behavior depended on the concentration, molar feed ratio and grafting density of the hydrogel precursors.

2.2.5. Stereocomplexes

2.2.5.1. Theoretical background. A polymer stereocomplex has been defined by Slager and Domb as 'a stereoselective interaction between two complementing stereoregular polymers, that interlock and form a new composite, demonstrating altered physical properties in comparison to the parent polymers' (Slager and Domb, 2003). The earliest reports on stereocomplexation date from 1953, when Pauling and Corey described association between enantiomeric D-and L-polypeptides (Pauling and Corey, 1953). In 1958 Fox et al. were the first to report on the formation of polymer stereocomplexes between syndiotactic and isotactic poly(methyl methacrylate) (PMMA) (Fox et al., 1958). Since then, numerous polymers, mainly enantiomers, have been described to form stereocomplexes (Slager et al., 2003). This extensive review also summarizes the multiple methods available for detection and characterization. In 1987 the formation of stereocomplexes between poly(L-lactide) (PLLA) and poly(D-lactide) (PDLA), exhibiting a 50 °C higher melting point than the individual enantiomers, was described by Ikada et al. (1987). Another decade later, the mechanism of stereocomplex formation between PLLA and PDLA was elucidated (Brizzolara et al.,

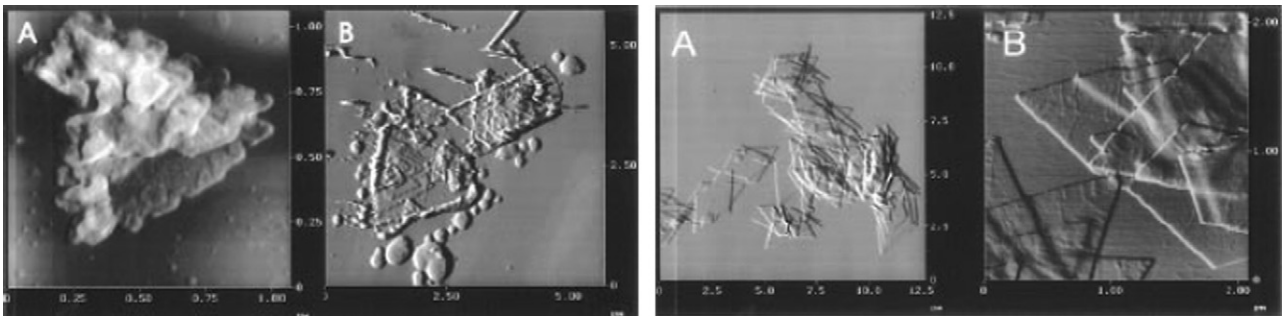

Fig. 14. AFM images of triangularly shaped stereocomplex single crystals (two left panels) and lozenge-shaped PDLA single crystals (two right panels) (reprinted from Brizzolara et al. (1996), with permission from ACS publications).

1996). It was found that a racemic mixture of PLLA and PDLA, in a 1:1 ratio, crystallizes into triangularly shaped 3_1-helices, whereas the homopolymers form lozenge-shaped 10_3-helices (Fig. 14). In the stereocomplex the 3_1-helices are stabilized by van der Waals interactions between opposite oxygen and hydrogen atoms, resulting in a higher melting point.

Furthermore, Brizzolara et al. suggested a possible crystal growing mechanism. It was postulated that a triangular nucleus of, *e.g.* PDLA is formed with exclusively PLLA to the sides. Next, a PDLA layer grows on the crystal surface after which another PLLA layer is formed, and so on (Fig. 15).

An elaborate review on poly(lactide) stereocomplexes and their formation, structure, properties and degradation, written by Tsuji, also includes the possible applications of these systems (Tsuji, 2005). Besides its use in films, fibers and microspheres, PLA is particularly interesting in self-assembling hydrogels, especially because of the possibility to create stereocomplex interactions.

2.2.5.2. Stereocomplexed hydrogels. De Jong et al. described physically crosslinked dextran hydrogels based on stereocomplexation between oligolactates of opposite chirality (De Jong et al., 2000) (Figs. 1F and 16).

Rheological analysis revealed that upon mixing aqueous solutions of dex-L-lactate and dex-D-lactate, an almost elastic hydrogel was formed, while a dex-L-lactate solution behaved like a viscoelastic material. X-ray diffraction was used to confirm the presence of stereocomplex crystals, creating the physical junctions between the dextran chains (De Jong et al., 2002). It was further found that when oligolactate chains of DP_{av} 5 were used, *i.e.* on average 5 repeating lactate units, only a weak network was formed upon mixing of dex-L- and dex-D-lactate, comparable to a dex-L-lactate solution alone. By increasing the DP_{av}, as well as the lactate substitution degree (DS) and the solid content of the gels (*i.e.* the initial polymer fraction in the gel) stronger hydrogels could be obtained. In the case of monodisperse oligomers, coupled to dextran, the oligolactate DP should at least be 11 to create hydrogels (De Jong et al., 2001). Degradation of the gels at pH 7 and 37 °C varied at pH 7 from 1 to 3.5 days for hydrogels of 70% water content, DP_{av} 9, DS 3 and DP_{av} 12 DS 6, respectively. A higher pH resulted in accelerated degradation whereas at pH 4 the hydrogels remained stable for more than 1 month. Van Nostrum et al. reported on a stereocomplexed hydrogel in which the dextran backbone was replaced by 2-hydroxypropyl methacrylamide (HPMA) (Van Nostrum et al., 2004). In these hydrogels, the oligolactate side chains

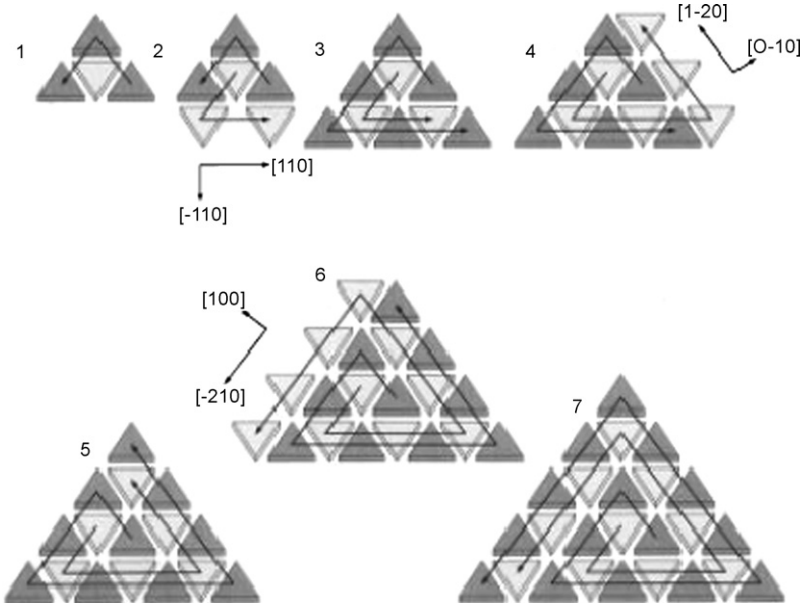

Fig. 15. Schematic growing mechanism of the stereocomplex single crystal (reprinted from Brizzolara et al. (1996), with permission from ACS Publications).

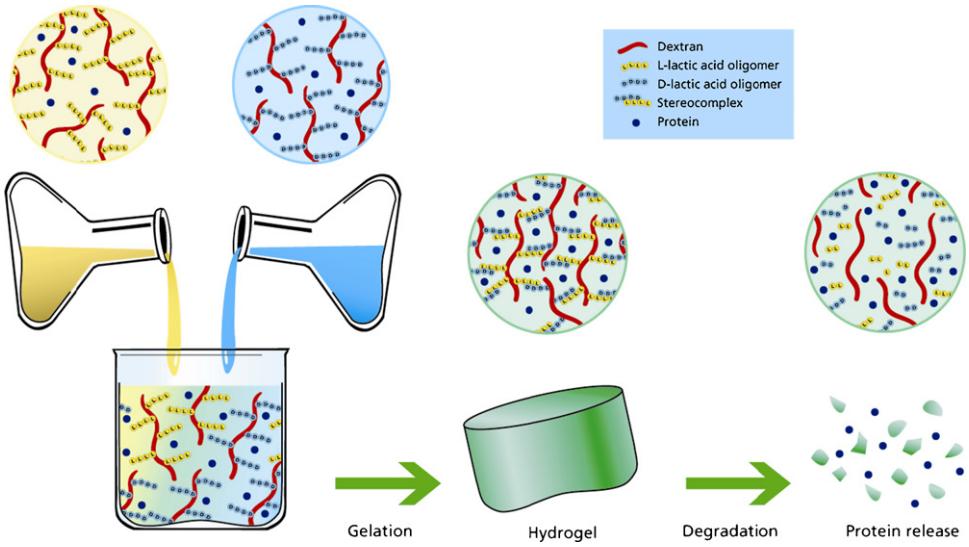

Fig. 16. Schematic presentation of the concept of the self-assembling stereocomplexed dextran hydrogel (reprinted from (De Jong, 2001)).

were acetylated, preventing rapid chain end scission (backbiting) making slow random chain scission the main action of degradation, leading to longer degradation times up to approximately 60 days. The release of model proteins (lysozyme and IgG) as well as the therapeutically relevant protein recombinant human interleukin-2 (rhIL-2) from the dex-lactate hydrogels was studied both *in vitro* and *in vivo*. A diffusion-controlled release of lysozyme during approximately 5 days was observed, whereas the larger protein IgG was mainly released subsequent to degradation of the matrix in about 8 days, from hydrogels with 70% initial water content and DS 6 (De Jong et al., 2001). RhIL-2 was rapidly released *in vitro* followed by a more gradual release the next days, with 65% of the protein released within 3 days, from hydrogels containing 82% water (Bos et al., 2004). Furthermore Bos et al. reported on the *in vivo* release of rhIL-2, which is a broad acting T cell-derived cytokine with anti-tumor activity, from dex-lactate hydrogels in SL2 lymphosarcoma-bearing mice. Dex-L-lactate and dex-D-lactate solutions, both containing rhIL-2, were mixed prior to injection in the peritoneal cavity where they gelled *in situ*. All mice treated with the rhIL-2-loaded gel, were cured, whereas the mice in the negative control groups (buffer and empty hydrogel) all died and the mice in the positive control groups (free rhIL-2, 1 and 5 injections) had a cure rate of 60% (Fig. 17). By the day the hydrogels were injected, the tumor had infiltrated the abdominal muscles and metastasized in lungs, liver and other organs, proving the systemic effect of the local rhIL-2 treatment. All cured mice appeared to be immune to the tumor after a rechallenge with SL2 cells at day 60 of the study. This study showed that the therapeutic efficacy of one injection of the rhIL-2-containing hydrogels was as least as good as the free rhIL-2 injections on 5 consecutive days. In a subsequent paper, Bos et al. also reported on the *in vivo* biocompatibility and tissue reactions of the dex-lactate gels after subcutaneous implantation in rats (Bos et al., 2005). It was demonstrated that the stereocomplexed dex-lactate hydrogels showed a good biocompatibility, evoking only a mild foreign body reaction, mainly directed to the degradation of the gels. The low number of lymphocytes indicated that the immune system was hardly triggered by the gel or degradation products.

Lim et al. reported on the synthesis and characterization of stereocomplexed hydrogels, composed of enantiomeric oligolactates coupled to pHEMA (Lim et al., 2000). The pendant oligo-L-lactate and oligo-D-lactate chains were able to interact and physical crosslinks were formed. It was shown that the hydrogels containing stereocomplex crystallinities degraded slower than materials with optically pure crystalline domains. The degradation rate could be varied by modifying the degree of polymerization (DP) of the oligolactates.

Fijiwara et al. synthesized PLLA-PEG-PLLA and PDLA-PEG-PDLA triblock copolymers by ring-opening polymerization of L-lactide or D-lactide in the presence of PEG and stannous 4-ethylhexanoate as a catalyst (Fujiwara et al., 2001). It was found that dispersions of single enantiomers remained fluid upon heating, whereas mixtures of both enantiomers formed a transparent gel at 37 °C and a white gel at 75 °C. This temperature-dependent sol–gel transition was ascribed to stereocomplex formation between the PLA chains of opposite chirality coupled to different PEG chains. The presence of stereocomplex crystallinities was confirmed by wide-angle X-ray scattering

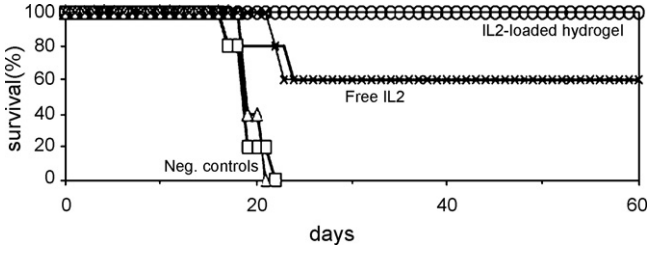

Fig. 17. Therapeutic efficacy in SL2-tumor-bearing mice of i.p. administered *in situ* gelled dex-lactate hydrogels loaded with rhIL-2 (DP_{av} = 12, DS = 6, water content = 82%): (△) ammonium acetate buffer pH 5.5; (□) empty hydrogel; (x) free rhIL-2 (1×10^6 IU, one injection) and free rhIL-2 (0.2×10^6 IU per injection, five injections); (◯) rhIL-2-loaded (1×10^6 IU) *in situ* hydrogel. Each group consisted of five mice (reprinted from Bos et al. (2004), with permission from Elsevier).

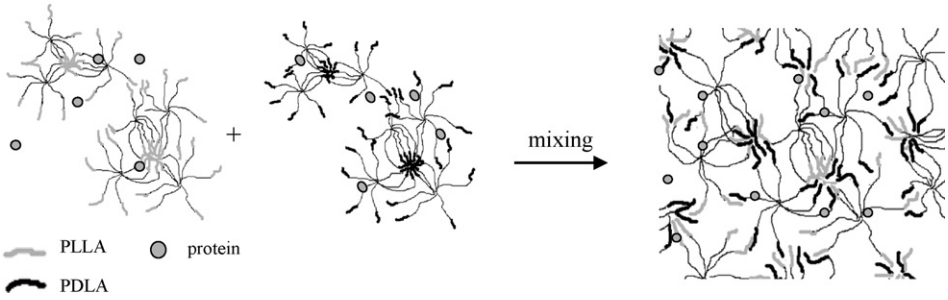

Fig. 18. Protein-loaded stereocomplexed hydrogels based on PEG-(PLLA)$_8$ and PEG-(PDLA)$_8$ star block copolymers (reprinted from Hiemstra et al. (2006c), with permission from Elsevier).

(WAXS). Fujiwara et al. hypothesized that this hydrogel might be interesting for biomedical applications, since gelation is slow and finishes around body temperature.

Similarly, Li and Vert synthesized diblock and triblock copolymers of PEG and PLLA/PDLA by ring-opening polymerization of L(D)-lactide in the presence of mono- or di-hydroxyl PEG with zinc metal as a catalyst (Li and Vert, 2003). Rheological analysis confirmed the formation of hydrogels by interaction of the enantiomeric PLLA and PDLA blocks. The presence of stereocomplexes was verified by Raman spectroscopy and WAXS. Gelation showed to be time- and temperature-dependent and was faster at 37 °C than at 20 °C. Li et al. pointed out that these systems could be very promising for the sustained release of drugs.

Hiemstra et al. reported on a novel *in situ* gelling system based on stereocomplexed star block copolymers (Hiemstra et al., 2006a). Ring-opening polymerization of either L-lactide or D-lactate in the presence of eight-arm PEG and a catalyst, lead to the formation of PEG-(PLLA)$_8$ and PEG-(PDLA)$_8$ star block copolymers. Upon mixing of aqueous solutions of equimolar amounts of both copolymers, in a concentration range of 5–25% (w/v) and 6–8% (w/v) for PEG 21,800 and PEG 43,500, respectively, self-assembly and hydrogel formation occurred. Wide-angle X-ray scattering confirmed the presence of stereocomplexes, the driving force for gelation. The hydrogel strength was increased while the gelation time decreased with increasing polymer concentration and PLLA/PDLA block length. In succeeding studies, the potential of these injectable hydrogels as protein delivery matrices was evaluated *in vitro* as well as *in vivo* (Hiemstra et al., 2006b; Hiemstra et al., 2007c). Protein loading of the hydrogels could be easily achieved by mixing aqueous protein solutions with the enantiomer solutions (Fig. 18). Lysozyme was quantitatively released in 10 days, following first order kinetics, with full preservation of its enzymatic activity. IgG release followed nearly zero-order release with 50% release in 16 days.

In a subsequent paper, Hiemstra et al. chemically modified the PEG-(PLLA)$_8$/PEG-(PDLA)$_8$ hydrogels in order to combine physical entanglements with photopolymerization (Hiemstra et al., 2007d). For this purpose, PEG-PLLA and PEG-PDLA star block copolymers were functionalized with methacrylate groups, either at the PLA chain ends (Fig. 1H) or at the PEG chain ends (Fig. 1I). It was found that the methacrylates hardly interfered with the stereocomplexation. On the other hand, after UV-polymerization, the hydrogels showed significantly higher storage moduli and prolonged degradation times when compared to only photopolymerized gels. Additionally, very low photoinitiator concentrations were needed when stereocomplexation had already occurred. Interestingly, hydrogels in which the methacrylates were coupled to the PEG ends degraded much slower than those gels in which the PLA ends where methacrylated. This novel approach circumvents the need for rapid UV-polymerization after injection of the hydrogels. Moreover, degradation times can be tailored by varying the degree and place of methacrylation, dependent on the foreseen application.

Recently, Van Tomme et al. described the formation of almost fully elastic hydrogels based on hydrophobic and stereocomplex interactions between oligolactate-grafted dextran microspheres (Van Tomme et al., 2008). Aqueous dispersions of microspheres substituted with L-oligolactates, combined with D-oligolactate-grafted microspheres, resulted in gels with highest strength, likely due to stereocomplexation between the enantiomers on the surface of various microspheres. The network properties could be modulated by varying the solid content of the gel, the DS and the DP of the oligolactate chains. Lysozyme release experiments showed a continuous release, with 50% released after 5 days and full preservation of the enzymatic activity of the entrapped protein. The biocompatible nature of the material, the protein-friendly self-assembly of the hydrogel (simply mixing the microspheres with protein solution) and the possibility to tailor the gel properties, makes this hydrogel system promising for various pharmaceutical applications.

3. Conclusions

The growing importance of hydrogels in tissue engineering and protein delivery applications has led to the development of many novel and promising preparation strategies. The overview of the systems described above, provided in Table 1, illustrates the diversity of *in situ* gelling systems that are currently being researched. While some approaches have already been tested on their biocompatibility and *in vivo* potential, others are still in their infancy. Whether some of the systems will make it to the clinic will depend on their *in vivo* performance in specific applications. Biocompatibility and biodegradability are indispensible properties. Additionally, when protein delivery is the foreseen application, a protein-friendly preparation technology and tailorable release behavior will be crucial. Scaffolds for tis-

sue engineering applications, on the other hand, will need to exhibit a custom-made degradation behavior. As outlined in this review, those hydrogels that are formed *in situ*, at the site of injection, preferably by self-assembly of the building blocks, show most potential for further development. Therefore, it can be foreseen that biomaterial research for biomedical applications will keep on focusing on these self-assembling hydrophilic systems.

References

Anseth, K.S., Metters, A.T., Bryant, S.J., Martens, P.J., Elisseef, J.H., Bowman, C.N., 2002. In situ forming degradable networks and their application in tissue engineering and drug delivery. J. Control. Release 78, 199–209.

Bailey, F.E., Koleske, J.V., 1976. Poly(ethylene oxide). Academic Press, New York.

Balakrishnan, B., Jayakrishnan, A., 2005. Self-cross-linking biopolymers as injectable in situ forming biodegradable scaffolds. Biomaterials 26, 3941–3951.

Berger, J., Mayer, J.M., Felt, O., Gurny, R., 2004a. Structure and interactions in chitosan hydrogels formed by complexation or aggregation for biomedical applications. Eur. J. Pharm. Biopharm. 57, 35–52.

Berger, J., Reist, M., Mayer, J.M., Felt, O., Peppas, N.A., Gurny, R., 2004b. Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. Eur. J. Pharm. Biopharm. 57, 19–34.

Bos, G.W., Jacobs, J.J.L., Koten, J.W., Van Tomme, S.R., Veldhuis, T.F.J., van Nostrum, C.F., Den Otter, W., Hennink, W.E., 2004. In situ crosslinked biodegradable hydrogels loaded with IL-2 are effective tools for local IL-2 therapy. Eur. J. Pharm. Sci. 21, 561–567.

Bos, G.W., Hennink, W.E., Brouwer, L.A., den Otter, W., Veldhuis, T.F.J., van Nostrum, C.F., van Luyn, M.J.A., 2005. Tissue reactions of in situ formed dextran hydrogels crosslinked by stereocomplex formation after subcutaneous implantation in rats. Biomaterials 26, 3901–3909.

Brack, H.P., Tirmizi, S.A., Risen, W.M., 1997. A spectroscopic and viscometric study of the metal ion-induced gelation of the biopolymer chitosan. Polymer 38, 2351–2362.

Brizzolara, D., Cantow, H.J., Diederichs, K., Keller, E., Domb, A.J., 1996. Mechanism of the stereocomplex formation between enantiomeric poly(lactide)s. Macromolecules 29, 191–197.

Bromberg, L.E., Ron, E.S., 1998. Temperature-responsive gels and thermogelling polymer matrices for protein and peptide delivery. Adv. Drug Deliv. Rev. 31, 197–221.

Choi, H.S., Yui, N., 2006. Design of rapidly assembling supramolecular systems responsive to synchronized stimuli. Prog. Polym. Sci. 31, 121–144.

Choi, H.S., Kontani, K., Huh, K.M., Sasaki, S., Ooya, T., Lee, W.K., Yui, N., 2002. Rapid induction of thermoreversible hydrogel formation based on poly(propylene glycol)-grafted dextran inclusion complexs. Macromol. Biosci. 2, 298–303.

Choi, H.S., Yamamoto, K., Ooya, T., Yui, N., 2005. Synthesis of poly(ε-lysine)-grafted dextrans and their pH- and thermosensitive hydrogelation with cyclodextrins. Chem. Phys. Chem. 6, 1081–1086.

Cohen, S., Lobel, E., Trevgoda, A., Peled, Y., 1997. A novel in situ-forming ophtalmic drug delivery system for alginates undergoing gelation in the eye. J. Control. Release 44, 201–208.

De Jong, S., 2001. Stereocomplex Formation of Lactic Acid Oligomers for the Design of Biodegradable Hydrogels. Department of Pharmaceutics, Utrecht, Utrecht University.

De Jong, S.J., De Smedt, S.C., Wahls, M.W.C., Demeester, J., Kettenes-van den Bosch, J.J., Hennink, W.E., 2000. Novel self-assembled hydrogels by stereocomplex formation in aqueous solution of enantiomeric lactic acid oligomers grafted to dextran. Macromolecules 33, 3680–3686.

De Jong, S.J., van Eerdenbrugh, B., van Nostrum, C.F., Kettenes-van den Bosch, J.J., Hennink, W.E., 2001. Physically crosslinked dextran hydrogels by stereocomplex formation of lactic acid oligomers: degradation and protein release behavior. J. Control. Release 71, 261–275.

De Jong, S.J., van Nostrum, C.F., Kroon-Batenburg, L.M.J., Kettenes-van de Bosch, J.J., Hennink, W.E., 2002. Oligolactate-grafted dextran hydrogels: detection of stereocomplex crosslinks by X-ray diffraction. J. Appl. Polym. Sci. 86, 289–293.

diZerega, G.S., Campeau, J.D., 2001. Peritoneal repair and post-surgical adhesion formation. Hum. Reprod. Update 7, 547–555.

Draye, J.-P., Delaey, B., Van de Voorde, A., Van Den Bulcke, A., Bogdanov, B., Schacht, E., 1998a. In vitro release characteristics of bioactive molecules from dextran dialdehyde cross-linked gelatin hydrogel films. Biomaterials 19, 99–107.

Draye, J.-P., Delaey, B., Van de Voorde, A., Van Den Bulcke, A., De Reu, B., Schacht, E., 1998b. In vitro and in vivo biocompatibility of dextran dialdehyde cross-linked gelatin hydrogel films. Biomaterials 19, 1677–1687.

Fedorovich, N.E., Alblas, J., De Wijn, J.R., Hennink, W.E., Verbout, A.J., Dhert, W.J.A., 2007. Hydrogels as extracellular matrices for skeletal tissue engineering: state of the art and novel application in organ printing—a review. Tissue Eng. 13, 1905–1925.

Fox, T.G., Garrett, B.S., Goode, W.E., Gratch, S., Kincaid, J.F., Spell, A., Stroupe, J.D., 1958. Crystalline polymers of methyl methacrylate. J. Am. Chem. Soc. 80, 1768–1769.

Fujiwara, T., Mukose, T., Yamaoka, T., Yamane, H., Sakurai, S., Kimura, Y., 2001. Novel thermo-responsive formation of a hydrogel by stereocomplexation between PLLA-PEG-PLLA and PDLA-PEG-PDLA block copolymers. Macromol. Biosci. 1, 204–208.

Goessl, A., Tirelli, N., Hubbell, J.A., 2004. A hydrogel system for stimulus-responsive, oxygen-sensitive in situ gelation. J. Biomater. Sci. Polym. Ed. 15, 895–904.

Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., Thom, D., 1973. Biological interactions between polysaccharides and divalent cations: the egg box model. FEBS Lett. 32, 195–198.

Haines-Butterick, L., Rajagopal, K., Branco, M., Salick, D., Rughani, R., Pilarz, M., Lamm, M.S., Pochan, D.J., Schneider, J.P., 2007. Controlling hydrogelation kinetics by peptide design for three-dimensional encapsulation and injectable delivery of cells. PNAS 104, 7791–7796.

Hamley, I.W., Ansari, I.A., Castelletto, V., Nuhn, H., Rosler, A., Klok, H.A., 2005. Solution self-assembly of hybrid block copolymers containing poly(ethylene glycol) and amphiphilic β-strand peptide sequences. Biomacromolecules 6, 1310–1315.

Hatefi, A., Amsden, B., 2002. Biodegradable injectable in situ forming drug delivery systems. J. Control. Release 80, 9–28.

He, P., Davis, S.S., Illum, L., 1998. In vitro evaluation of the mucoadhesive properties of chitosan microspheres. Int. J. Pharm. 166, 75–88.

Hennink, W.E., van Nostrum, C.F., 2002. Novel crosslinking methods to design hydrogels. Adv. Drug Deliv. Rev. 54, 13–36.

Hiemstra, C., Zhong, Z., Li, L., Dijkstra, P.J., Feijen, J., 2006a. In-situ formation of biodegradable hydrogels by stereocomplexation of PEG-$(PLLA)_8$ and PEG-$(PDLA)_8$ star block copolymers. Biomacromolecules 7, 2790–2795.

Hiemstra, C., Zhong, Z.Y., Van Tomme, S.R., Hennink, W.E., Dijkstra, P.J., Feijen, J., 2006b. Protein release from injectable stereocomplexed hydrogels based on PEG-PDLA and PEG-PLLA star block copolymers. J. Control. Release 116, e19–e21.

Hiemstra, C., Zhong, Z.Y., Jiang, X., Hennink, W.E., Dijkstr, P.J., Feyen, J., 2006c. PEG-PLLA and PEG-PDLA multiblock copolymers: synthesis and *in situ* hydrogel formation by stereocomplexation. J. Control. Release 116, e17–e19.

Hiemstra, C., vanderAa, L.J., Zhong, Z., Dijkstra, P.J., Feijen, J., 2007a. Novel in situ forming, degradable dextran hydrogels by Michael Addition chemistry: synthesis, rheology, and degradation. Macromolecules 40, 1165–1173.

Hiemstra, C., vanderAa, L.J., Zhong, Z., Dijkstra, P.J., Feijen, J., 2007b. Rapidly in situ-forming degradable hydrogels from dextran thiols through Michael Addition. Biomacromolecules 8, 1548–1556.

Hiemstra, C., Zhong, Z., Van Tomme, S.R., van Steenbergen, M.J., Jacobs, J.J.L., Otter, W.D., Hennink, W.E., Feijen, J., 2007c. In vitro and in vivo protein delivery from in situ forming poly(ethylene glycol)-poly(lactide) hydrogels. J. Control. Release 119, 320–327.

Hiemstra, C., Zhou, W., Zhong, Z., Wouters, M., Feijen, J., 2007d. Rapidly in situ forming biodegradable robust hydrogels by combining stereocomplexation and photopolymerization. J. Am. Chem. Soc. 129, 9918–9926.

Hill-West, J.L., Chowdhury, S.M., Slepian, M.J., Hubbell, J.A., 1994. Inhibition of thrombosis and intimal thickening by in situ photopolymerization of thin hydrogel barriers. Proc. Natl. Acad. Sci. 91, 5967–5971.

Hoffman, A.S., 2002. Hydrogels for biomedical applications. Adv. Drug Deliv. Rev. 43, 3–12.

Huang, X., Lowe, T.L., 2005. Biodegradable thermoresponsive hydrogels for aqueous encapsulation and controlled release of hydrophilic model drugs. Biomacromolecules 6, 2131–2139.

Huang, X., Nayak, B.R., Lowe, T.L., 2004. Synthesis and characterization of novel thermoresponsive-co-biodegradable hydrogels composed of *N*-isopropylacrylamide, poly (L-lactic acid), and dextran. J. Polym. Sci. Part A: Polym. Chem. 42, 5054–5066.

Huh, K.M., Ooya, T., Lee, W.K., Sasaki, S., Kwon, I.C., Jeong, S.Y., Yui, N., 2001. Supramolecular-structured hydrogels showing a reversible phase transition by inclusion complexation between poly(ethylene glycol) grafted dextran and α-cyclodextrin. Macromolecules 34, 8657–8662.

Ikada, Y., Jamshidi, K., Tsuji, H., Hyon, S.H., 1987. Stereocomplex formation between enantiomeric poly(lactides). Macromolecules 20, 904–906.

Ito, T., Yeo, Y., Highley, C.B., Bellas, E., Kohane, D.S., 2007. Dextran-based in situ cross-linked injectable hydrogels to prevent peritoneal adhesions. Biomaterials 28, 3418–3426.

Jeong, B., Bae, Y.H., Lee, D.S., Kim, S.W., 1997. Biodegradable block copolymers as injectable drug-delivery systems. Nature 388, 860–862.

Jeong, B., Bae, Y.H., Kim, S.W., 2000. In situ gelation of PEG-PLGA-PEG triblock copolymer aqueous solutions and degradation thereof. J. Biomed. Mater. Res. 50, 171–177.

Jeong, B., Kim, S.W., Bae, Y.H., 2002. Thermosensitive sol–gel reversible hydrogels. Adv. Drug Deliv. Rev. 54, 37–51.

Jiang, Z., You, Y., Deng, X., Hao, J., 2007. Injectable hydrogels of poly([epsilon]-caprolactone-*co*-glycolide)-poly(ethylene glycol)-poly([epsilon]-caprolactone-*co*-glycolide) triblock copolymer aqueous solutions. Polymer 48, 4786–4792.

Jin, R., Hiemstra, C., Zhong, Z., Feijen, J., 2007. Enzyme-mediated fast in situ formation of hydrogels from dextran-tyramine conjugates. Biomaterials 28, 2791–2800.

Kashyap, N., Viswanad, B., Sharma, G., Bhardwaj, V., Ramarao, P., Ravi Kumar, M.N.V., 2007. Design and evaluation of biodegradable, biosensitive in situ gelling system for pulsatile delivery of insulin. Biomaterials 28, 2051–2060.

Klouda, L., Mikos, A.G., 2008. Thermoresponsive hydrogels in biomedical applications. Eur. J. Pharm. Biopharm. 68, 34–45.

Kopecek, J., 2007. Hydrogel biomaterials: a smart future? Biomaterials 28, 5185–5192.

Kopecek, J., Yang, J., 2007. Hydrogels as smart biomaterials. Polym. Int. 56, 1078–1098.

Kotzé, A.F., Luessen, H.L., Thanou, M.M., Verhoef, J.C., de Boer, A.G., Junginger, H.E., Lehr, C.M., 1999. Chitosan and chitosan derivatives as absorption enhancers for peptide drugs across mucosal epithelia. In: Mathiowitz, E., Chickering, D.E., Lehr, C.M. (Eds.), Bioadhesive Drug Delivery Systems. Marcel Dekker Inc., New York, pp. 341–385.

Kretlow, J.D., Klouda, L., Mikos, A.G., 2007. Injectable matrices and scaffolds for drug delivery in tissue engineering. Adv. Drug Deliv. Rev. 59, 263–273.

Kuo, C.K., Ma, P.X., 2001. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering. Part 1. Structure, gelation rate and mechanical properties. Biomaterials 22, 511–521.

Leach, J.B., Schmidt, C.E., 2005. Characterization of protein release from photocrosslinkable hyaluronic acid-polyethylene glycol hydrogel tissue engineering scaffolds. Biomaterials 26, 125–135.

Lee, J., Bae, Y.H., Sohn, Y.S., Jeong, B., 2006. Thermogelling aqueous solutions of alternating multiblock copolymers of poly(L-lactic acid) and poly(ethylene glycol). Biomacromolecules 7, 1729–1734.

Li, S., Vert, M., 2003. Synthesis, characterization, and stereocomplex-induced gelation of block copolymers prepared by ring-opening polymerization of L(D)-lactide in the presence of poly(ethylene glycol). Macromolecules 36, 8008–8014.

Lim, D.W., Choi, S.H., Park, T.G., 2000. A new class of biodegradable hydrogels stereocomplexed by enantiomeric oligo(lactide) side chains of poly(HEMA-g-OLA)s. Macromol. Rapid Commun. 21, 464–471.

Mahler, A., Reches, M., Rechter, M., Cohen, S., Gazit, E., 2006. Rigid, self-assembled hydrogel composed of a modified aromatic dipeptide. Adv. Mater. 18, 1365–1370.

Maia, J., Ferreira, L., Carvalho, R., Ramos, M.A., Gil, M.H., 2005. Synthesis and characterization of new injectable and degradable dextran-based hydrogels. Polymer 46, 9604–9614.

Mi, F.-L., Shyu, S.-S., Wong, T.-B., Jang, S.-F., Lee, S.-T., Lu, K.-T., 1999. Chitosan-polyelectrolyte complexation for the preparation of gel beads and controlled release of anticancer drug. II. Effect of pH-dependent ionic crosslinking or interpolymer complex using tripolyphosphate or polyphosphate as reagent. J. Appl. Polym. Sci. 74, 1093–1107.

Monteiro, O.A.C., Airoldi, C., 1999. Some studies of crosslinking chitosan-glutaraldehyde interaction in a homogeneous system. Int. J. Biol. Macromol. 26, 119–128.

Muzzarelli, R.A.A., 1997. Human enzymatic activities related to the therapeutic administration of chitin derivatives. Cell. Mol. Life Sci. (CMLS) 53, 131–140.

Nguyen, K.T., West, J.L., 2002. Photopolymerizable hydrogels for tissue engineering applications. Biomaterials 23, 4307–4314.

Ossipov, D.A., Brännvall, K., Forsberg-Nilsson, K., Hilborn, J., 2007. Formation of the first injectable poly(vinyl alcohol) hydrogel by mixing of functional PVA precursors. J. Appl. Polym. Sci. 106, 60–70.

Packhaeuser, C.B., Schnieders, J., Oster, C.G., Kissel, T., 2004. In situ forming parenteral drug delivery systems: an overview. Eur. J. Pharm. Biopharm. 58, 445–455.

Pauling, L., Corey, R.B., 1953. Two rippled-sheet configurations of polypeptide chains, and a note about the pleated sheets. PNAS 39, 253–256.

Peppas, N.A., 1997. Hydrogels and drug delivery. Curr. Opin. Colloid Interface 2, 531–537.

Peppas, N.A., Bures, P., Leobandung, W., Ichikawa, H., 2000. Hydrogels in pharmaceutical formulations. Eur. J. Pharm. Biopharm. 50, 27–46.

Pochan, D.J., Schneider, J.P., Kretsinger, J., Ozbas, B., 2003. Thermally reversible hydrogels via intramolecular folding and consequent self-assembly of a de novo designed peptide. J. Am. Chem. Soc. 125, 11802–11803.

Qiu, B., Stefanos, S., Ma, J., Lalloo, A., Perry, B.A., Leibowitz, M.J., Sinko, P.J., Stein, S., 2003. A hydrogel prepared by in situ cross-linking of a thiol-containing poly(ethylene glycol)-based copolymer: a new biomaterial for protein drug delivery. Biomaterials 24, 11–18.

Ruel-Gariepy, E., Leroux, J.-C., 2004. In situ-forming hydrogels—review of temperature-sensitive systems. Eur. J. Pharm. Biopharm. 58, 409–426.

Salgado-Rodriguez, R., Licea-Claverie, A., Arndt, K.F., 2004. Random copolymers of *N*-isopropylacrylamide and methacrylic acid monomers with hydrophobic spacers: pH-tunable temperature sensitive materials. Eur. Polym. J. 40, 1931–1946.

Sato, T., Ishii, T., Okahata, Y., 2001. In vitro gene delivery mediated by chitosan. Effect of pH, serum, and molecular mass of chitosan on the transfection efficiency. Biomaterials 22, 2075–2080.

Sawhney, A.S., Pathak, C.P., Hubbell, J.A., 1993. Bioerodible hydrogels based on photopolymerized poly(ethylene glycol)-*co*-poly(alpha-hydroxy acid) diacrylate macromers. Macromolecules 26, 581–587.

Sawhney, A.S., Pathak, C.P., van Rensburg, J.J., Dunn, R.C., Hubbell, J.A., 1994. Optimization of photopolymerized bioerodible hydrogel properties for adhesion prevention. J. Biomed. Mater. Res. 28, 831–838.

Schacht, E.H., Bogdanov, B., Van Den Bulcke, A., De Rooze, N., 1997. Hydrogels prepared by crosslinking of gelatin with dextran dialdehyde. React. Funct. Polym. 33, 109–116.

Slager, J., Domb, A.J., 2003. Biopolymer stereocomplexes. Adv. Drug Deliv. Rev. 55, 549–583.

Smeds, K.A., Grinstaff, M.W., 2001. Photocrosslinkable polysaccharides for in situ hydrogel formation. J. Biomed. Mater. Res. 55, 254–255.

Tae, G., Kim, Y.J., Choi, W.I., Kim, M., Stayton, P.S., Hoffman, A.S., 2007. Formation of a novel heparin-based hydrogel in the presence of heparin-binding biomolecules. Biomacromolecules 8, 1979–1986.

Tanna, S., Joan Taylor, M., Sahota, T.S., Sawicka, K., 2006. Glucose-responsive UV polymerised dextran-concanavalin A acrylic derivatised mixtures for closed-loop insulin delivery. Biomaterials 27, 1586–1597.

Tsuji, H., 2005. Poly(lactide) stereocomplexes: formation, structure, properties, degradation and applications. Macromol. Biosci. 5, 569–597.

Van Nostrum, C.F., Veldhuis, T.F.J., Bos, G.W., Hennink, W.E., 2004. Tuning the degradation rate of poly(2-hydroxypropyl methacrylamide)-graft-oligo(lactid acid) stereocomplex hydrogels. Macromolecules 37, 2113–2118.

Van Tomme, S.R., Hennink, W.E., 2007. Biodegradable dextran hydrogels for protein delivery applications. Expert Rev. Med. Devices 4, 147–164.

Van Tomme, S.R., De Geest, B.G., Braeckmans, K., De Smedt, S.C., Siepmann, F., Siepmann, J., van Nostrum, C.F., Hennink, W.E., 2005a. Mobility of model proteins in hydrogels composed of oppositely charged dextran microspheres studied by protein release and fluorescence recovery after photobleaching. J. Control. Release 110, 67–78.

Van Tomme, S.R., van Steenbergen, M.J., De Smedt, S.C., van Nostrum, C.F., Hennink, W.E., 2005b. Self-gelling hydrogels based on oppositely charged dextran microspheres. Biomaterials 26, 2129–2135.

Van Tomme, S.R., van Nostrum, C.F., De Smedt, S.C., Hennink, W.E., 2006. Degradation behavior of dextran hydrogels composed of positively and negatively charged microspheres. Biomaterials 27, 4141–4148.

Van Tomme, S.R., Mens, A., van Nostrum, C.F., Hennink, W.E., 2008. Macroscopic hydrogels by self-assembly of oligolactate-grafted dextran microspheres. Biomacromolecules 9, 158–165.

Veerman, C., Rajagopal, K., Palla, C.S., Pochan, D.J., Schneider, J.P., Furst, E.M., 2006. Gelation kinetics of β-hairpin peptide hydrogel networks. Macromolecules 39, 6608–6614.

Vermonden, T., Besseling, N.A.M., van Steenbergen, M.J., Hennink, W.E., 2006. Rheological studies of thermosensitive triblock copolymer hydrogels. Langmuir 22, 10180–10184.

West, J.L., Hubbell, J.A., 1995. Photopolymerized hydrogel materials for drug delivery applications. React. Polym. 25, 139–147.

Wichterle, O., Lim, D., 1960. Hydrophilic gels for biological use. Nature 185, 117–118.

Xu, C., Breedveld, V., Kopecek, J., 2005. Reversible hydrogels from self-assembling genetically engineered protein block copolymers. Biomacromolecules 6, 1739–1749.

Yu, Y.B., 2002. Coiled-coils: stability, specificity, and drug delivery potential. Adv. Drug Deliv. Rev. 54, 1113–1129.

Yu, L., Chang, G.T., Zhang, H., Ding, J.D., 2008. Injectable block copolymer hydrogels for sustained release of a PEGylated drug. Int. J. Pharm. 348, 95–106.

Zentner, G.M., Rathi, R., Shih, C., McRea, J.C., Seo, M., Oh, H., Rhee, B.G., Mestecky, J., Moldoveanu, Z., Morgan, M., Weitman, S., 2001. Biodegradable block copolymers for delivery of proteins and water-insoluble drugs. J. Control. Release 72, 203–215.

Zhang, X.-Z., Wu, D.-Q., Sun, G.-M., Chu, C.-C., 2003. Novel biodegradable and thermosensitive Dex-AI/PNIPAAm hydrogel. Macromol. Biosci. 3, 87–91.

Zheng Shu, X., Liu, Y., Palumbo, F.S., Luo, Y., Prestwich, G.D., 2004. In situ crosslinkable hyaluronan hydrogels for tissue engineering. Biomaterials 25, 1339–1348.