2

Biodegradable dextran hydrogels for protein delivery applications

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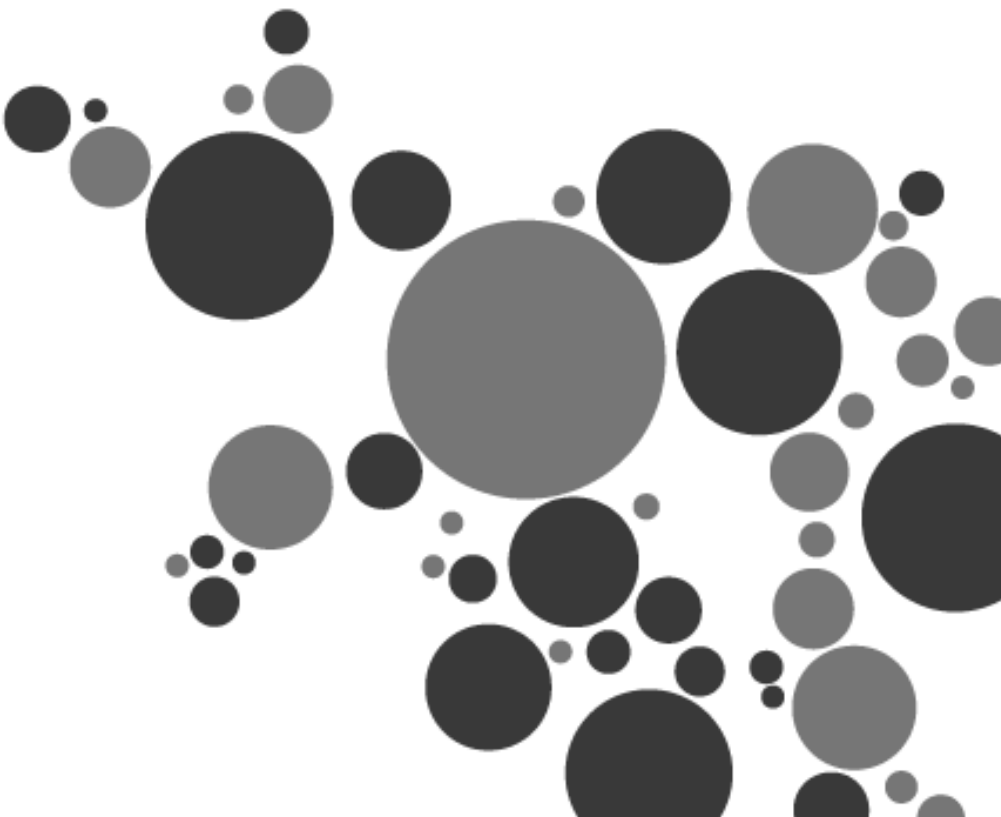

Summary

The rapid development of protein-based pharmaceuticals during the past decades has tremendously increased the need for suitable delivery systems, guaranteeing a safe and controlled delivery of proteinacous drugs. Hydrogels offer good opportunities as protein delivery systems or tissue engineering scaffolds because of an inherent biocompatibility. Their hydrophilic, soft and rubbery nature ensures minimal tissue irritation and a low tendency of cells and proteins to adhere to the hydrogel surface. A variety of both natural and synthetic polymers have been used for the design of hydrogels in which network formation is established by chemical or physical crosslinking. This review will introduce the general features of hydrogels and will further on focus on dextran hydrogels in particular. Chemically and physically crosslinked systems will be described and their potential suitability as protein delivery systems as well as tissue engineering scaffolds will be discussed. Special attention will be given to network properties, protein delivery, degradation behavior and biocompatibility.

Keywords: hydrogels, dextran, chemical crosslinking, physical crosslinking, protein delivery, tissue engineering, biodegradation, biocompatibility

1. Introduction

Nobel Prize winner Paul Berg's invention of recombinant DNA in 1972 opened the door to a whole new world of biopharmaceuticals.[1] Indeed, modern biotechnology made it possible to produce virtually any protein that could be of therapeutic use in a variety of diseases, including cancer, immune disorders and infectious diseases. The number of recombinant proteins entering clinical studies over the past 30 years is enormous and it is predicted that their number will keep on growing the next decade. In 2004 and 2005 the FDA approved 16 new protein therapeutics.[2] However, even more than the traditional drug molecules, these novel therapeutics do suffer from a lack of suitable delivery systems.[3] Proteins are fragile molecules sensitive to both physical degradation induced by, among others, pH and temperature and chemical degradation, such as oxidation or enzymatic cleavage. Denatured and degraded proteins do not only endure a loss of biological activity, more importantly, they might induce an immune response.[4] Therefore, a thorough understanding of protein stability, immunogenicity and pharmacokinetic profile is indispensable to develop a successful protein-based therapeutic.[5] Oral delivery, the most preferred, non-invasive route of administration is unattainable due to the harsh conditions in the gastrointestinal tract. In the stomach, low pH and enzymatic degradation affect the protein integrity. Additionally, proteins have difficulties to pass the intestinal epithelium. After parenteral administration, most proteins are rapidly cleared from the circulation, requiring the need of frequent injections or continuous infusions. In order to extend the plasma half-life of proteins and increase their safety and efficacy by avoiding peak concentrations and prolonging the presence in specific tissues and organs, specific formulation strategies such as controlled release systems, are needed. Alternatively, conjugation of proteins and peptides with poly(ethylene glycol) (PEG) has also shown to lead to improved clinical properties such as increased solubility and stability, extended circulation time and reduced immunogenicity and susceptibility to enzymatic degradation.[6] As a result of sustained release or prolonged circulation of proteins, the injection frequency is reduced and patient comfort and compliance are increased.[7, 8]

Polymeric systems such as hydrogels,[9, 10] microspheres[11] and nanoparticles,[12] as well as lipid-based systems such as liposomes[13] and water-in-oil emulsions[14] have been studied extensively for the delivery of pharmaceutical peptides and proteins. This review will focus on biodegradable hydrogels, in particular dextran-based systems, designed for protein delivery and tissue engineering applications.

2. Hydrogels

Hydrogels are three-dimensional hydrophilic matrices, capable of absorbing large quantities of water. They have been used in a variety of applications, *e.g.* as wound dressings, transdermal patches, drug delivery devices, contact lenses or in reconstructive surgery. Their soft and rubbery nature provokes minimal tissue irritation and makes them particularly attractive to incorporate proteins and cells. Natural (*e.g.* collagen,[15] hyaluronate,[16] alginate,[17] starch,[18] chitosan[19]) as well as synthetic polymers (*e.g.* poly(ethylene glycol),[20] poly(vinyl alcohol),[21] poly(hydroxyethyl methacrylate)[22]) can be used to prepare hydrogels, of which the latter are better defined and

provide more control over physical and chemical properties. Additionally, natural polymers can be derived from various sources with possible contaminations as a result. Depending on the application not only biocompatibility, *i.e.* the ability of a material to perform with an appropriate host response in a specific application,[23] but also biodegradability will be requested. The degradation properties under physiological conditions can be tailored by incorporation of degradable linkers.[10, 24] Physical and chemical methods can be applied to obtain crosslinking of polymers into hydrogel structures in order to prevent dissolution of the hydrophilic polymer chains in the aqueous environment. Chemical crosslinking can be accomplished by radical polymerization, high-energy irradiation, via enzymes or by chemical reaction of complementary groups. These methods result in a network with a relatively high mechanical strength and, depending on the nature of the chemical bonds in the building blocks and the crosslinks, in tailor-made degradation times.[25] However, chemical crosslinking can possibly damage the entrapped bioactive substance, leading to a loss of activity. Moreover, the crosslinking agents are mostly toxic and removal needs to be ensured before *in vivo* application. In recent years there is a growing interest in physically crosslinked hydrogels. In these systems non-permanent bonds, based on physical interactions between the polymer chains, such as ionic, hydrophobic or antigen-antibody interactions, hydrogen bonding and crystallization, are created.[25] An attractive class of physically crosslinked systems is those where gel formation occurs after a certain trigger (*e.g.* temperature, pH, ionic strength).[26, 27] These stimuli-responsive hydrogels, as well as those that are formed after a certain time (*e.g.* stereocomplexes[28]) or after UV-irradiation,[29] are particularly interesting as injectable, *in situ*-forming matrices for drug delivery and tissue engineering applications.[30-34] Table 1 summarizes the properties an 'ideal' hydrogel should have to be suitable as protein delivery system or as tissue engineering scaffold. As will be discussed in the next section, dextran meets most of these requirements, making it an attractive polymer to design hydrogels.

Table 1: 'Ideal' properties of hydrogels suitable for biomedical applications.

Protein delivery system	Tissue engineering scaffold
Biocompatible and biodegradable	
Injectable	
Self-assembling	
Non-toxic degradation products	
Mild inflammatory reaction	
Non-immunogenic	
Tailorable release properties	Tailorable mechanical strength
Possibility to target specific sites	Tailorable degradation time
	Controllable shape and size

3. Dextran based hydrogels

Table 2 gives an overview of the various strategies developed to create dextran hydrogels. The possible formulations as well as the specific *in vitro* and *in vivo* evaluations carried out are listed.

Table 2: Overview of dextran derivatives, crosslinking methods, formulation options and type of *in vitro/in vivo* evaluations carried out.

Polymer	X-linking	Formulations	*In vitro*	*In vivo*	Ref
Chemical crosslinking					
Dex-MA Dex-HEMA	Radical polymerization (KPS/TEMED)	Hydrogel implants Microspheres Scaffolds	IgG and rhIL-2 release Tissue regeneration	Release of rhIL-2 and hGH Biocompatibility	39-54
Functionalized dextran (*e.g.* sulfate)	STMP	Particles	rhTGF-β1 and rhBMP-2 release Cell support	Biocompatibility	55-56
CM-dextran	EDC/NHS	Membranes	pH and ionic strength dependent drug transport		57
Dex-AI or dex-MA copolymerized with NiPAAm	UV-polymerization	Thermosensitive hydrogels and Particles			58-61
Dex-AE copolymerized with PEGDA	UV-polymerization	pH-sensitive hydrogel implants	BSA release		62
Dex-MA copolymerized with con A-MA	UV-polymerization	Glucose-responsive hydrogel implants	Insulin release		63-64
Oxidized dextran Oxidized dextran + gelatin	AAD	Hydrogels Hydrogel films	EGF release	Biocompatibility	65 66-68
Dex-DVA	Enzyme	Hydrogel implants		Biocompatibility	69-70
Physical crosslinking					
Dextran 6000	Crystallization	Hydrogels Microspheres			71
Dex-PEG or dex-PPG + cyclodextrins	Hydrogen bonding	Temperature sensitive hydrogels			72-73
Dex-PLL + cyclodextrins	Hydrogen bonding	Temperature and pH-sensitive hydrogels			74
Dex-L-lactate + dex-D-lactate	Stereo-complexes	Injectable hydrogels	Lysozyme, IgG and rhIL-2 release	rhIL-2 release Biocompatibility	27, 75-80
Dextran-sulfate + chitosan	Ionic interactions	Nanoparticles	Insulin release		81

3.1. Introduction

Dextran is an exocellular bacterial polysaccharide predominantly consisting of linear α-1,6-linked glucopyranose units, with some degree of 1,3-branching (Fig. 1A). This highly water-soluble polymer is produced in a sucrose-rich environment by *Lactobacillus*, *Leuconostoc* and *Streptococcus* and is commercially available with different molecular weights. Both the degree of branching and the molecular weight distribution affect the physicochemical properties.[35] Native dextran has a high molecular weight and a high degree of polydispersity, which can be decreased by partial hydrolysis and subsequent fractionation.[36]

Dextrans with an average molecular weight of 1000 to 2 million g/mol are commercially available for research purposes.[37] Clinically, dextran, in particular low molecular weight (40000 and 70000 g/mol), has been used for over 50 years in plasma volume expansion, thrombosis prophylaxis, peripheral blood flow enhancement and for the rheological improvement of, for instance, artificial tears.[36, 37] After parenteral administration, low molecular weight dextran (40000 g/mol or smaller) has a half-life of 8 hours and is secreted by the kidneys.[38] Dextrans of a higher molecular weight exhibit longer half-lives and are subsequently degraded by the reticuloendothelial system.[39] Additionally, dextrans are metabolized by different dextranases (α-1-glucosidases) in various parts of the body, including liver, spleen and colon.[36]
Besides their favorable characteristic of being highly water-soluble, dextrans are stable under mild acidic and basic conditions. Furthermore, these polymers contain a large number of hydroxyl groups, making them suitable for derivatization and subsequent chemical or physical crosslinking.[36]
The past decades research interest has focused on the use of dextran as macromolecular carriers, *e.g.* hydrogels, in which the drug can be incorporated. Dextran hydrogels can be obtained in various ways, based on either chemical or physical crosslinking (as listed in Table 2).

3.2. Chemically crosslinked dextran hydrogels

3.2.1. Methacrylate-derivatized dextrans

Edman *et al.* pioneered in the research on polymerizable dextran by reaction of dextran with glycidylacrylate in water.[40] Hydrogels were formed after addition of the initiator system *N,N,N',N'*-tetramethylethylenediamine (TEMED) and ammonium peroxydisulfate (APS) to an aqueous solution of acryldextran in the presence of *N,N*-methylenebisacrylamide. Enzyme-loaded microspheres were obtained using an emulsion polymerization technique during which the enzyme activity was completely retained. In our department methacrylate-derivatized dextrans (dex-MA) (Fig. 1B) have been crosslinked in the presence of potassium peroxydisulfate (KPS) and TEMED as initiating system, leading to hydrogels, insensitive to hydrolysis at physiological conditions.[41] Introduction of hydrolysis-sensitive bonds between the dextran backbone and the methacrylate side chains resulted in hydrogels that are fully biodegradable under physiological conditions. In this way hydroxyethyl methacrylate-derivatized dextran (dex-HEMA) (Fig. 1C) hydrogels were created in which carbonate esters are the hydrolyzable units. Additional control over the degradation profile was obtained after incorporation of a lactate spacer (dex-lactate-

HEMA) (Fig. 1D).[42] The degradation time could be varied from one day to more than three months, depending on the crosslink density, water content, type of ester group in the crosslinks and length of the lactate spacer. Both the *in vitro* and *in vivo* biocompatibility and biodegradability of these dextran hydrogels has been demonstrated and it was shown that the *in vivo* degradation rate correlates well to the *in vitro* situation.[43, 44] Not only macroscopic gels but also microspheres could be prepared from these dextran derivatives. An all-aqueous preparation method was developed, based on the phase separation between PEG and dextran solutions.[45, 46]

Controlled release of a model protein (IgG) from enzymatically degrading macroscopic dex-MA hydrogels could be accomplished by incorporation of an endo-dextranase, responsible for degradation of the dextran backbone.[47] The release was dependent on the crosslink density of the gels and the amount of dextranase incorporated. Release of IgG from dex-HEMA gels with a low crosslink density (DS 3, degree of substitution, *i.e.* number of HEMA side chains per 100 glucopyranose units) and moderate water content (70 % (w/w)) occurred over 20 days in a nearly zero-order fashion. Hydrogels with a higher DS and lower water content exhibited a delayed release, of which the delay time was dependent on the crosslink density of the gels (*e.g.* 35 days for DS 11). Eventually, around 65 % of the entrapped IgG was released after 15 days. Release of IgG from dex-HEMA microspheres also showed a delay time, however, considerably shorter than for macroscopic gels. Interestingly, a zero-order IgG release from enzymatically degrading dex-MA microspheres was observed, while the dex-HEMA microspheres exhibited a biphasic release profile. The authors state that these differences might result from the fact that during degradation of dex-MA microspheres, the dextran backbone is enzymatically hydrolyzed, while in dex-HEMA microspheres, hydrolysis of the side chains is the cause of degradation. In macroscopic hydrogels on the other hand, the protein remains entrapped as long as the microscopic cages are not yet connected with each other, resulting in a delayed release from both dex-MA and dex-HEMA gels.[48] Besides model proteins the release of therapeutically relevant proteins from dextran hydrogels was also studied. The release of the recombinant human cytokine interleukin-2 (IL-2), an important mediator of the immune response, was investigated by Cadée *et al.*[49] Release from the non-degradable dex-MA gels occurred in a diffusion-controlled fashion and was affected by the water content and crosslink density of the network. HPLC and radioactivity measurements showed that rhIL-2 was not quantitatively released with 6 and 80 % of the initial amount still present after 35 days in gels with a water content of 90 and 70 %, respectively. Decreasing the water content of degradable dex-lactate-HEMA and dex-HEMA gels also decreased the rhIL-2 release rate. The release from highly hydrated dex-lactate-HEMA gels (90 % water content) followed Fickian diffusion. Gels with a water content of 70 % or lower, released the protein in an almost zero-order profile during 5 to 15 days. Cadée *et al*. attribute this release profile to two factors compensating each other: firstly, the diffusion coefficient of the protein increases due to swelling of the matrix in time, secondly, the hydrogel size increases resulting in a decreased concentration gradient and a decreased release rate. RhIL-2 release from dex-HEMA gels was shown to be slower than from dex-lactate-HEMA gels with comparable network characteristics, ascribed to a faster degradation of the dex-lactate-HEMA gels. Importantly, it was shown that rhIL-2 was mainly released in its monomeric form from all the gel types and maintained 50 to 70 % of its original activity. Release of rhIL-2 from non-biodegradable dex-MA and biodegradable dex-HEMA microspheres was studied *in vivo* in tumor-bearing mice by de Groot *et al.*[50] The required amount of rhIL-2 was gradually released over

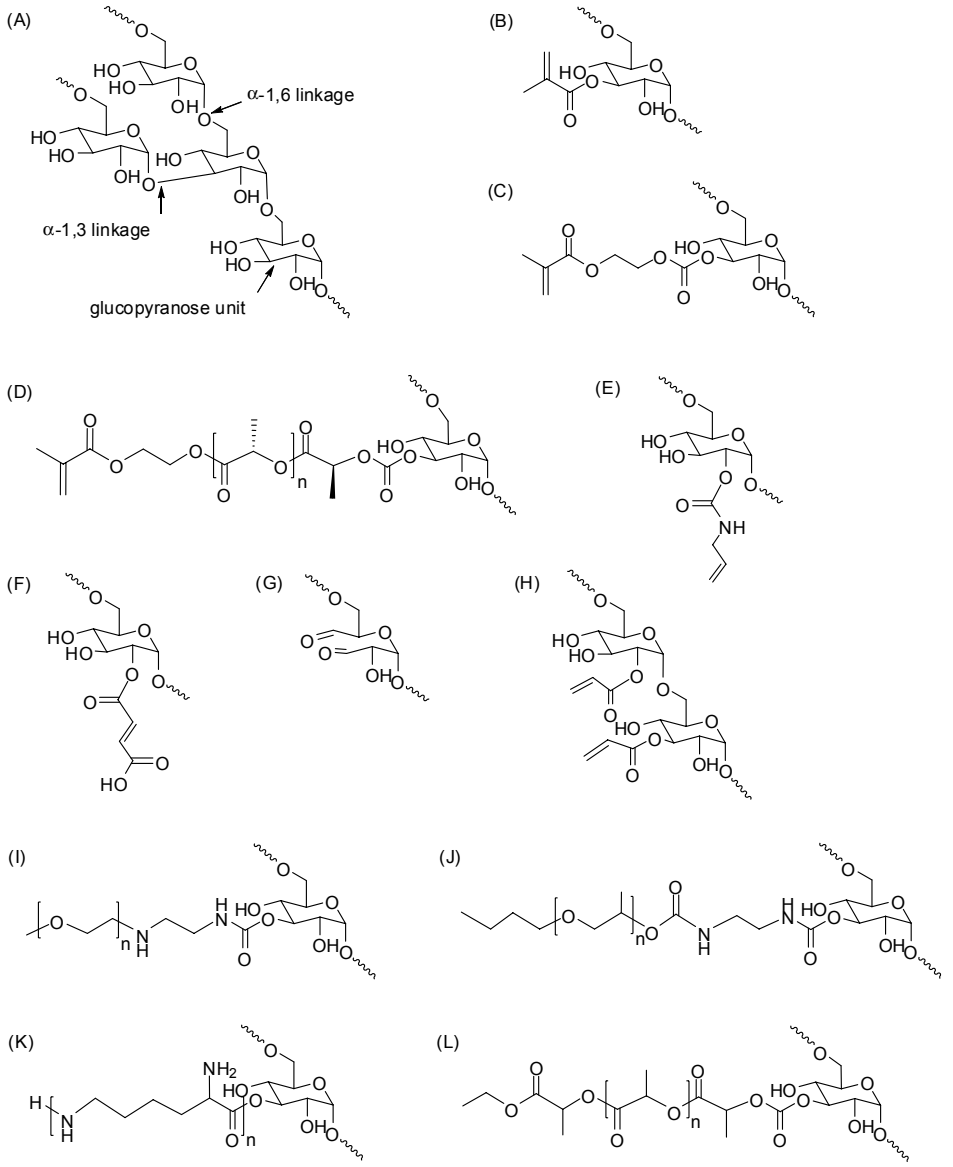

Figure 1: Building blocks of various dextran-based hydrogels:
(A) dextran (B) dex-MA (methacrylate) (C) dex-HEMA (D) dex-lactate-HEMA (E) dex-AI (F) dex-MA (maleic acid) (G) oxidized dextran (H) dex-DVA (I) dex-PEG (J) dex-PPG (K) dex-PL (L) dex-lactate.

a period of 5 to 10 days. The therapeutic effect of one injection of rhIL-2-containing microspheres was comparable to the effect of free rhIL-2 injections for 5 consecutive days. Vlugt-Wensink *et al.* studied the release of human growth hormone (hGH) from dex-HEMA microspheres subcutaneously injected in Dwarf mice and healthy human volunteers.[51] In dwarf mice, a single injection of the hGH-containing microspheres resulted in a significant, dose-dependent increase in body length and weight. Daily injection of a single dose hGH gave the same results, indicating the hGH released from the microspheres was fully bioactive. Administration of hGH-loaded microspheres to human healthy volunteers led to increased hGH serum concentrations from day 2 on, with peak concentrations after 7-8 days. The serum concentration of the biomarkers insulin-like growth factor-I (IGF-1) and IGF binding protein response-3 (IGFBP-3) followed the hGH profile, again demonstrating that the released hGH was bioactive. Additionally, a good *in vitro - in vivo* correlation of the hGH release was found.

De Geest *et al.* used charged microspheres based on dex-HEMA copolymerized with methacrylic acid (MAA) or dimethylamino ethyl methacrylate (DMAEMA) as templates for layer-by-layer (LbL) assembly of the polyelectrolytes poly(sodium 4-styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) for the design of self-rupturing microcapsules for pulsed drug delivery (Fig. 2).[52, 53] Sequential adsorption of polyelectrolytes led to membranes that could be controlled within nanometers. It was found that the permeability of the coating was pH dependent. Three bilayers of PSS/PAH rendered the microcapsules impermeable to 20000 g/mol FITC-dextran at pH 9 while they were still permeable at pH 7. Six polyelectrolyte bilayers were needed to make the membranes impermeable to 20000 g/mol FITC-dextran at pH 7. Upon degradation of the microgels at pH 9, dextran degradation products of 19000 g/mol are formed, unable to penetrate the surrounding membrane. It was found that sudden rupture of the membrane occurred as a result of an increased osmotic pressure due to the presence of degradation products inside the capsule. The self-rupturing of the microcapsules is completely controlled by the degradation kinetics of the gels, independent of any external stimulus. Self-exploding dextran-based microcapsules appear to be promising systems for pulsed delivery of antigens for vaccination purposes. However, further research is needed to evaluate the applicability of the system under physiological conditions.

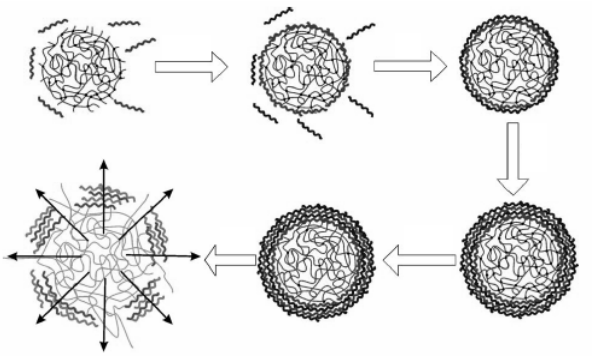

Figure 2: Schematic presentation of the coating, swelling and explosion of a dex-HEMA microgel. Reprinted from Journal of Controlled Release 116, 159-169 (2006) De Geest *et al.* with permission from Elsevier.[52]

Lévesque *et al.*[54] reported on the use of dex-MA for the preparation of hydrogel scaffolds, also taking advantage of the immiscibility of aqueous solutions of PEG and dextran (Fig. 3).[45] Dex-MA was polymerized in glass molds in the presence of an excess of water and PEG by which porous structures were formed. It was found that the concentration of PEG dramatically influenced the pore structure of the scaffolds, ranging from microporous to macroporous gel-wall to macroporous with interconnected beads. It was possible to predict the porosity of the scaffolds by making use of dex-MA/PEG/H_2O phase diagrams. The authors pointed out that macroporous gel scaffolds with interconnected beaded structure can be used in tissue engineering since cell penetration, nutrient diffusion and tissue regeneration are facilitated. Recently, the same research group described macroporous scaffolds of dex-MA copolymerized with aminoethyl methacrylate (AEMA).[55] The primary amines were introduced to allow grafting of extracellular matrix (ECM)-derived peptides on the dex-MA-co-AEMA hydrogels to promote specific cellular interactions. It was found that the introduction of primary amines (AEMA) as such, without additional grafting of ECM-derived peptides, improved cell adhesion already. Importantly, modification of the scaffolds with peptides further enhanced the cell adhesion and moreover a significant increase in cellular activity, as measured by neurite outgrowth, was observed.

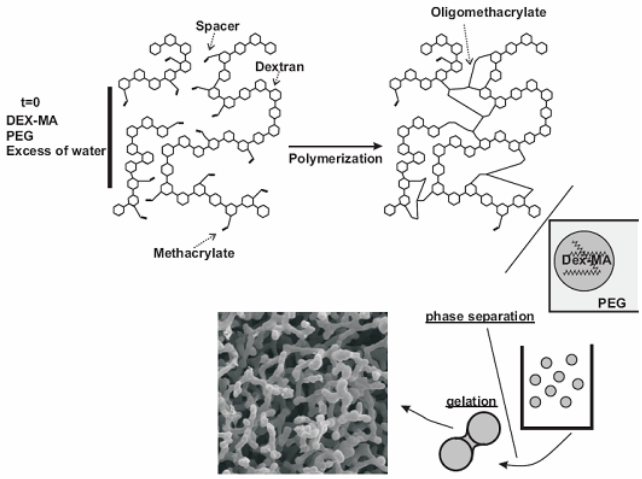

Figure 3: Preparation of dex-MA scaffolds making use of the PEG/dextran phase separation. Reprinted from Biomaterials 26, 7436-7446 (2005) Lévesque *et al.* with permission from Elsevier.[53]

3.2.2. Functionalized dextrans bearing negatively charged groups

Functionalized dextrans (FD), bearing carboxylate, benzylamide and sulfate groups, were described by Maire *et al.* as potential building blocks for hydrogels which are able to bind and release transforming growth factor β1 (TGF-β1).[56] Hydrogels were obtained through chemical crosslinking of dextran and FD with sodiumtrimetaphosphate (STMP) as a crosslinking agent under alkaline conditions. After drying, the hydrogels were crushed and sieved to obtain particles of 1.0 to 1.6 mm in diameter. Protein loading was achieved by soaking of the particles in buffer containing rhTGF- β1 for 30 min at 4 °C. Negatively charged phosphate groups, produced during

the crosslinking reaction as well as the negative charge of the FD resulted in electrostatic binding of rhTGF-β1 to the hydrogel matrix. A burst release, ascribed to a rapid desorption of the protein was observed during the first six hours, after which the protein was slowly released over 3 days. The porous particles provided a high exchange surface leading to a fast release. Furthermore, loading of hydrogels by soaking in protein solution mostly results in an inhomogeneous distribution of the protein in the matrix and subsequent burst release. The retaining capacity of the gels was dependent on the FD and crosslinker content. In another study the functionalized dextran-based hydrogels were evaluated as bone morphogenic protein (BMP) carriers to enhance bone formation.[57] The material is injectable making it suitable for repair of irregularly shaped skeletal defects. In view of this application a relatively long BMP retention capacity is important. Maire *et al.* found that rhBMP-2 was less retained than rhTGF-β1, investigated in the first study, due to a different interaction pattern (ion-pairing, Van der Waals, hydrogen bonding) between the proteins and the matrix. Nevertheless, significant bone formation was seen with only half of the amount of BMP required when compared to a collagen sponge. An irreproducible calcification of the hydrogels was observed *in vivo*, which was explained by interaction of the anionic chemical groups with Ca^{2+} ions, causing precipitation of phosphocalcic mineral structures. Calcification and bone formation occurred independently of each other but it was shown that calcification improved the biocompatibility of the hydrogels as evidenced by the absence of a major inflammatory reaction. A limitation of these scaffolds is their inability to allow cell ingrowth into the core of the system, solely leading to bone formation between the particles and at the exterior of the scaffold.

3.2.3. Stimuli-responsive dextran hydrogels

3.2.3.1. pH, ionic strength- and temperature-sensitive materials

A pH- and ionic strength-sensitive dextran hydrogel was developed by the group of Hubble.[58] Hydrogel formation was accomplished by the intermolecular crosslinking between hydroxyl and carboxyl groups of carboxymethyl (CM) dextran using 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide (EDC) in the presence of *N*-hydroxysuccinimide (NHS). The reaction of dextran and sodium chloroacetate, catalyzed by NaOH, was varied from 15 min at 62 °C to 1 h at 70 °C, to introduce sufficient crosslinkable groups to obtain a less or more pH-sensitive material, respectively. SEM images revealed a reversible pH-dependent morphology of the membranes, with a more compact interior structure at pH 5.5 than at pH 7.4. At higher pH the COOH groups dissociate, initiating electrostatic repulsion between the polymer chains and as a consequence increasing the porosity of the matrix. Consequently, diffusion of lysozyme through the hydrogel membranes at pH 5.5 was significantly slower than at pH 7.4 and the transport rate changed reversibly when the pH was switched repeatedly between acidic and neutral. The lysozyme transport also proved to be influenced by the ionic strength of the buffer, with the highest diffusion rate in ionic strengths of 0.15 M and 0.2 M at pH 5.5 and 7.4, respectively. A further increase in ionic strength led to a reduced lysozyme transport due to shielding of the COO^- groups by Na^+ ions resulting in a reduced swelling. Neither a pH- nor an ionic strength-dependency was observed when hydrogel membranes were used with a lower carboxylic acid substitution. As a result of a lower amount of COOH groups in the network, less repulsive forces are active with a

reduced swelling as a consequence. This offers the possibility to tailor the hydrogels for specific applications.

Copolymerization of NIPAAm with dextran derivatives was used by Zhang *et al.* to create thermosensitive hydrogels.[59, 60] Dextran was substituted with allyl isocyanate (dex-AI) (Fig. 1E) and photopolymerized with NIPAAm. Differential scanning calorimetry showed an increasing LCST (= lower critical solution temperature) from 32 to 36.8 °C when the amount of dextran moieties was increased from 0 to 50 % (w/w), as a result of a shift in the hydrophilic/hydrophobic balance. No LCST was found in the studied temperature range (25-50 °C) in hydrogels containing even more dextran units due to the extensive number of non-thermosensitive polymers. SEM analysis of freeze-dried hydrogels showed that the gels were denser when the dextran content increased from 20 to 80 %, with pore sizes from 3.5 to 0.8 μm, respectively. Due to the presence of the NIPAAm groups in the gels the swelling of the gels decreased when the temperature was elevated above the LCST. Furthermore, below the LCST, the swelling ratio was reduced when the dextran content was augmented, owing to a denser network, higher pore density and less thermosensitivity. Depending on specific requirements of possible applications, the response to external temperature changes of these gels can be tailored by varying the hydrophilic/hydrophobic balance of the gels.

The same group reported on temperature and pH-sensitive hydrogels, again composed of dextran and NIPAAm.[61] In this study, the dextran hydroxyl groups were derivatized with maleic anhydride, yielding a polymerizable macromer with carboxylic acid groups (Fig. 1F). As for the dex-AI/PNIPAAm systems, an LCST increase from 35.9 to 39.1 °C was observed when the ratio (w/w) dex-MA/PNIPAAm was increased from 0.25 to 4. These slightly higher LCST values when compared to the Dex-AI composites were due to a different hydrophilicity level of the dextran derivatives. SEM imaging revealed a change in pore structure from irregular round and loose to well-defined honey-comb like, when dex-MA was copolymerized with NIPAAM (Fig. 4). Increasing amounts of dex-MA resulted in smaller pores. As in the first study, Zhang *et al.* explain the relationship between pore size and dextran content by the presence of the crosslinkable groups along the dextran backbone, affecting the crosslinking level. A higher dextran content leads to more crosslinks and a denser network as a result. The crosslinking level also greatly affected the swelling ratio with less swelling with increasing dex-MA content. Moreover, the gel properties were pH dependent. In alkaline medium the gels exhibited a swelling ratio that was almost double of that in acidic medium. The free carboxylic groups in dex-MA are deprotonated at neutral and alkaline pH, causing electrostatic repulsion between the polymer chains and thus an increased swelling.

The dex-AI/PNIPAAm hybrid hydrogels were also used for the preparation of temperature-sensitive dendrite-shaped particles.[62] Microgels (average diameter of 1 mm) were obtained after precipitation polymerization of dex-AI and NIPAAm in water at 75 °C in the presence of ammonium persulfate (APS) as initiator. DSC measurements showed an LCST around 26 °C, which is significantly lower than the LCST of the corresponding macroscopic gels. Additionally a faster and larger swelling of the particles was observed when compared to macroscopic gels due to a lower crosslinking and higher surface area.

A major disadvantage of the PNIPAAm-containing hydrogels described above is the non-biodegradability of the thermosensitive polymer. Extensive *in vitro* and *in vivo* degradation studies need to be conducted to evaluate the biodegradability of the dex/PNIPAAm hybrid networks.

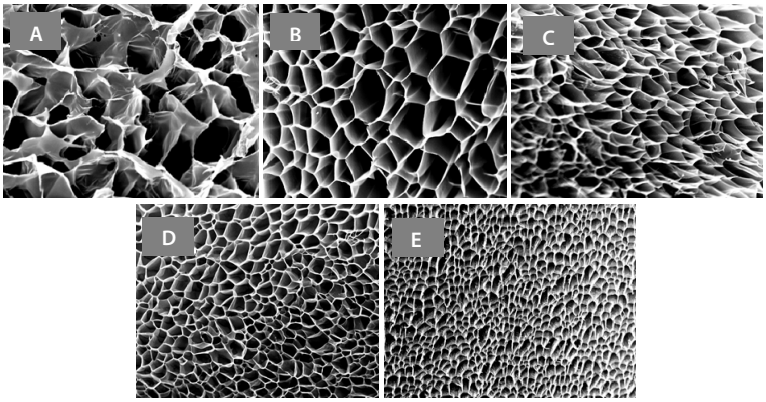

Figure 4: SEM images of dex-MA/PNIPAAm hydrogels:
Figure (A) is pure PNIPAAm. Figures (B) to (E) show hydrogels with increasing dextran content. Reprinted from Biomaterials 25, 4719-4730 (2004) Zhang *et al.* with permission from Elsevier.[60]

Sun *et al.* reported on a photopolymerized pH-sensitive hydrogel composed of dextran-allyl isocynate-ethylamine (dex-AE) and poly(ethylene glycol)-diacrylate (PEGDA)[63]. The influence of the dex-AE/PEGDA ratio on the pore size of the gels was investigated with SEM. A denser and more compact interior morphology was observed with increasing content of dex-AE from 0 to 30 %. Further increase of the dex-AE content to 50 and 70 % led to a more open and looser structure. This phenomenon was attributed to an incomplete crosslinking, also evidenced by a reduced conversion efficiency due to the lower activity of the C=C bonds in the allyl isocyanate of dex-AE when compared to the C=C bonds in PEGDA. Swelling studies confirmed these findings with a lowest swelling ratio for gels with the intermediate dex-AE content (30 %). Furthermore, the swelling ratio was pH dependent, with a higher swelling at pH 3 than at pH 10, especially significant for those hydrogels containing higher amounts of dex-AE. This effect was ascribed to electrostatic repulsion between the protonated amine groups in dex-AE in acidic conditions, leading to expansion of the network and increase in swelling. The hydrogels were loaded with BSA by soaking of the gels in BSA solution for 48 h at room temperature. Release of BSA took place over 10 to 30 days from hydrogels with 10 to 70 % dex-AE. All hydrogels exhibited a burst release during the first 8 h, attributed to release of BSA located near the surface of the gels, as a result of the inhomogeneous loading of the gels after soaking in BSA solution. Contrary to the results of the swelling experiments and the SEM images, a more sustained release was observed from gels with a higher dex-AE content. This indicates that the BSA release is not only dependent on the network structure but also on electrostatic interactions between the positively charged amine groups in dex-AE and negatively charged BSA molecules.

3.2.3.2. Glucose-responsive dextran hydrogels

Glucose-responsive UV-polymerized dextran-based hydrogels were developed by Tanna *et al.* [64] for the design of a self-regulating insulin delivery system. Dextran and concanavalin A (con A) were derivatized with methacrylic anhydride to introduce polymerizable groups. Con A is a glucose-selective lectin, *i.e.* a protein containing moieties that specifically interact with glucose. Rheological analysis of the crosslinked mixtures revealed that although there are covalent bonds present, the complex viscosity decreased with increasing glucose concentration. This effect can be attributed to the competition between free glucose and glucopyranose units of dextran to interact with specific receptor sites of con A. To be of physiological relevance, the system should have a reduced complex viscosity, enabling the release of insulin, when the glucose concentration is 0.1-1 %, which corresponds to ~ 5.5-55 mmol/L. Tanna *et al.* found that a dex-MA (DS 3)-con A-MA gel, which was irradiated with UV-light (365 nm, 10 mJ cm^{-2}) for 50 min, showed an 80 % drop in viscosity when the relevant glucose concentration was reached. A longer irradiation time resulted in more permanent crosslinks, leading to a viscosity drop of only 50 % for gels that were irradiated for 70 and 100 min. However, sufficient crosslinking is essential to minimize component leach, *i.e.* non-bonded con A and dex-MA. *In vitro* insulin release studies revealed an increasing graded response to glucose concentrations between 0.1 and 1 %. Additionally, insulin release was reversibly affected by the glucose concentration and could be triggered repeatedly (Fig. 5). In a subsequent paper Tanna *et al.* investigated the effect of the derivatization degree of both dextran and con A on the glucose-dependent insulin release.[65] As would be expected, the presence of more polymerizable acrylic groups on dextran led to a tighter crosslinked network and related material properties. Surprisingly, non-polymerized dextran and con A derivatives also showed a glucose-dependent complex viscosity profile. This was ascribed to the competition of glucose units in dextran and free glucose with the glucose-binding sites on con A, causing a rupture of the three-dimensional network. Apparently, besides covalent chemical crosslinks, physical entanglements are important for the network properties of the mixture. A slower glucose response time was found when the substitution degree of dextran was raised from 3 to 5.8 %. The derivatization degree of con A had an optimum at 60 %, with the lowest component leach and satisfactory glucose sensitivity. A higher substitution degree resulted in partial denaturation of con A leading to a loss of glucose binding capacity and less effective physical crosslinking with dextran chains. To design a closed-loop insulin delivery device, a compromise between the glucose response time and the glucose sensitivity of the material is indispensable. Therefore, the crosslink density of the hydrogel should be sufficient to prevent an early drop of the complex viscosity but the network should not hamper the insulin diffusion outwards.

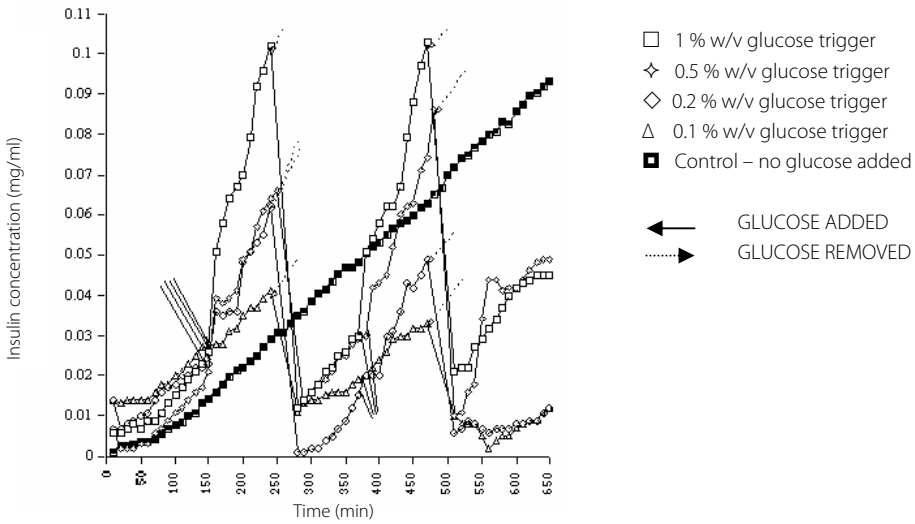

Figure 5: Insulin release from dex-MA-con A-MA hydrogels in response to altering glucose concentrations. Reprinted from Biomaterials 27, 1586-1597 (2006) Tanna *et al.* with permission from Elsevier.[63]

3.2.4. Oxidized dextrans

Maia *et al*. took advantage of the self-assembling properties of oxidized dextrans (dexOx) (Fig. 1G) in the presence of adipic acid dihydrazide (AAD) to form injectable hydrogels.[66] Dextran was oxidized with sodium periodate and subsequent reaction of the aldehyde groups with hydrazide groups of AAD resulted in crosslinking at room temperature overnight. Rapid conversion of a viscous solution to an elastic gel was observed within 4 minutes after which a long curing process (above 3 h) took place before complete gelation was accomplished. Swelling and degradation of the hydrogels was dependent on the number of intermolecular crosslinks and as a consequence on the AAD content. At physiological conditions, the degradation time could be varied from 9 to 15 and 23 days, for gels consisting of 15 % oxidized dextrans with, respectively, 5, 10 and 20 % of AAD. The influence of degradation on the pore-size was studied by means of SEM and mercury intrusion porosimetry (MIP). Maia *et al.* found that the pore size increased during degradation of the network from 1.5 to 6.3 µm after 11 days. In tissue engineering applications this might assist the ingrowth of cells and result in a better integration of the matrix with the surrounding tissue.

Hydrogels prepared by reaction of aldehyde groups of oxidized dextran with amino residues in gelatin were described by Schacht *et al.*[67] Both chemical crosslinks and physical gelation of gelatin were responsible for the gel strength. It was found that the storage modulus (G') increased with increasing degree of oxidation of dextran. An oxidation degree lower than 20 % resulted in weaker networks when compared to control gelatin hydrogels, probably due to partial separation between the incompatible gelatin and dextran phases. Sustained release of epidermal growth factor (EGF) from hydrogel films was observed up to 7 days.[68] Schacht *et al*. emphasize that the *in vivo* release profiles might differ considerably from the *in vitro* results due to the biodegradable nature of gelatin and dextran. *In vivo* biocompatibility studies of gelatin-oxidized dextran composites showed a moderate foreign body reaction around the subcutaneous implants.[69]

3.2.5. Enzymatically synthesized hydrogels

An elegant enzymatic synthetic route toward dextran hydrogels was developed by Ferreira *et al.* [70, 71] They made use of a single-step biocatalytic transesterification reaction between dextran and divinyladipate (DVA) in dimethylsulfoxide (DMSO) (Fig. 1H). Different proteases and lipases were tested on their conversion capability, of which an alkaline protease from *B. subtilis* (Proleather FG-F) performed the best. The elasticity modulus of the enzymatically produced hydrogels ranged from 1.4 kPa to 5.8 kPa for hydrogels with a degree of substitution (DS, *i.e.* number of DVA molecules per 100 glucopyranose residues) of respectively 20 and 47 %. Presumably, the Proleather catalysis favors the formation of intermolecular crosslinks due to a high efficiency in promoting the attachment of both of the terminal vinyl ester groups of DVA to dextran. Additionally, SEM images and MIP measurements of the biocatalytic hydrogels showed that the total porosity was at least 80 % with a unimodal, narrow and relatively homogenous pore size distribution with average diameters of 0.4 to 2 µm, depending on the crosslink density of the network. Histological examination of surrounding tissue after subcutaneous implantation of the hydrogels in rats indicated a good biocompatibility of the materials. The inflammatory response was mild with granulocyte and lymphocyte cells disappeared by day 10. Macrophages that had phagocytosed hydrogel particles were observed 5 to 40 days after implantation, depending on the DS. This phagocytosis process begins earlier for low DS hydrogels since less crosslinks are present and degradation is more rapid. Only for hydrogels with the highest DS (31 and 47 %) collagen, deposited by the fibroblasts surrounding the macrophages and foreign body giant cells, organized into a fibrous capsule by day 10. For the DS 31 % gel no mature capsule could be formed due to degradation of the gel by that time, while for the DS 47 % a discontinuous fibrous capsule was observed. Ferreira *et al.* state that these gels are suitable for tissue engineering and drug delivery applications because of their superior mechanical properties, no substantial fibrous capsule formation after implantation and porosities above 80 %.

3.3. Physically crosslinked dextran hydrogels

As pointed out above, hydrogels created by physical interactions are especially attractive candidates for protein delivery and tissue engineering applications since they are formed through self-assembly, without the aid of crosslinking agents.

3.3.1. Crystallization of dextran in aqueous solution

Crystallization was used by Stenekes *et al.* to produce physically crosslinked dextran hydrogels.[72] Sol-gel conversion of low-molecular weight dextran (Mw 6000 g/mol) in aqueous solution was observed caused by intermolecular hydrogen bonding. Rheology confirmed the formation of mainly elastic gels with tan(δ) values of 0.05 to 0.03 for 40 and 60 % dextran 6000 solutions, respectively. For more concentrated solutions a shorter lag time and a higher G' plateau value was observed, demonstrating that precipitation and gelation were more rapid. The authors hypothesize that the dextran chains associate through hydrogen bonding, after which nucleation and growth of the crystals occurs. A low degree of hydration in more concentrated solutions as well as the presence of salt ions that require water molecules to dissolve, facilitate association of

the chains and thus crystallization. Stirring of the solution induces orientation of the chains also resulting in accelerated crystallization.

3.3.2. Polymer inclusion complexes of cyclodextrins with dextran derivatives

The group of Yui reported on supramolecular assembly of hydrophobically aggregated crystalline domains creating injectable polymer inclusion complexes (PICs). They made use of the favorable property of cyclodextrins (CDs) to selectively include a wide range of guest molecules. CDs are cyclic water-soluble oligosaccharides with internal hydrophobic cavities. PEG[73] or poly(propylene glycol) (PPG),[74] of which it is known to form inclusion complexes with α-CDs and β-CDs, respectively, was grafted to dextran (Fig. 1I and 1J). Addition of the graft copolymers to a CD solution rendered the solution opaque en eventually led to the formation of a gel as a result of physical crosslinks between crystalline inclusion complexes, induced by hydrogen bonding between CDs threaded along the PEG/PPG chains. The time required for complete 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. Contrary, when PEG/PPG was only mixed with CDs, crystalline precipitates were formed, as a result of intermolecular hydrogen bonding between the CDs threaded along the PEG/PPG chains. When hydrophilic dextran chains are present, the hydrophobic PIC domains act as physical junctions, creating a hydrogel (Fig. 6). It was further found that above a certain temperature ($T_{gel\text{-}melting}$) the supramolecular assembly dissociates yielding a viscous solution, but after cooling down to a specific temperature ($T_{gelation}$) an opaque gel was reformed. Significant differences were observed between the $T_{gel\text{-}melting}$ and $T_{gelation}$ and both transitions could be controlled by altering the concentration, the molar ratio [EG/PG]/[CD] and the PEG/PPG content of the graft copolymer. For dex-PEG-α-CD complexes with a molar [EG]/[CD] ratio of 2 the $T_{gel\text{-}melting}$ and $T_{gelation}$ typically ranged from 50 to 60 °C and from 35 to 45 °C, respectively, depending on the PEG content. A dramatic increase of the $T_{gel\text{-}melting}$ and $T_{gelation}$ was found with an increasing [EG]/[CD] ratio from 1 to 2, but a further increase when the ratio was above 2 was hardly seen.[73]

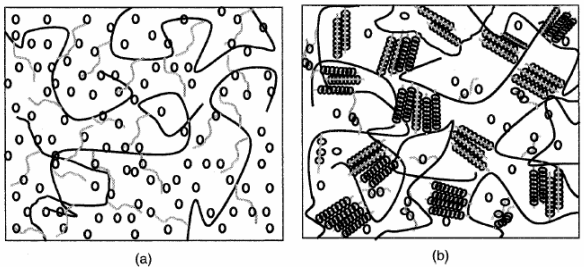

Figure 6: Schematic presentation of the self-assembly of dex-PEG with α-CDs into supramolecular structures. (A) Uncomplexed state before inclusion complexation occurs (B) α-CDs threaded along PEG chains resulting in inclusion complexes. Reprinted from Macromolecules 34, 8657-8662 (2001) Huh *et al.* with permission from ACS.[72]

For dex-PPG-β-CD complexes with a molar [PG]/[CD] ratio of 2 the $T_{gel\text{-}melting}$ and $T_{gelation}$ was 60 to 65 °C and 80 to 85 °C respectively, also depending on the PPG content (Fig. 7). Both $T_{gel\text{-}melting}$ and $T_{gelation}$ gradually decreased when the [PG]/[CD] ratio exceeded 2.[74] It is known that the stoichiometry of [EG/PG]/[CD] is 2, meaning that at higher ratios almost all the PEG/PPG grafts and CD moieties already participated in the inclusion complexation with no significant additional change in the gel properties as a result.

In addition to the thermoreversible gelation based on physical interactions between hydrophobic inclusion complexes a pH-sensitive functionality was introduced.[75] In this study dextran was grafted with a cationic polymer poly(ε-lysine) (PL) (Fig. 1K). At high pH, the primary amines of PL are deprotonated, allowing the CDs to be threaded onto the PL chain. Again, the concentration, molar feed ratio and grafting density were determining for the phase transition behavior. At pH 4, a rapid gel-to-sol transition occurred, due to dissociation of the inclusion complexes in the protonated state of PL. This is due to the energetically unfavorable situation of protonated amines in the hydrophobic cavities of the CDs.

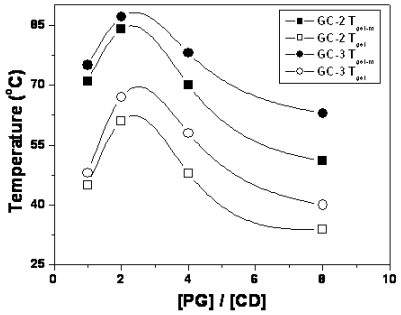

Figure 7: Melting and gelation temperatures of dex-PPG/β-CD mixtures as a function of the PG/CD ratio and the number of PPG graft. Reprinted from Macromolecular Bioscience 2, 298-303 (2002) Choi *et al.* with permission from Wiley Interscience.[73]

3.3.3. Stereocomplexation

Upon mixing of two polymer enantiomers, possessing opposite chirality, the formation of crystals occurs, referred to as stereocomplexes. De Jong *et al.* made use of stereocomplex formation to create physically crosslinked hydrogels.[76] Lactic acid oligomers were synthesized and coupled to dextran (Fig. 1L). Both L- and D-lactide were used resulting in dex-L- and dex-D-lactate. Rheological analysis revealed that upon mixing aqueous solutions of both polymers a hydrogel was formed. In time, a substantial increase of the elasticity modulus (G') was observed whereas the dex-L-lactate solutions did not show any change. Creep experiments showed an almost elastic behavior of the mixture while the dex-L-lactate solution behaved as a visco-elastic material (Fig. 8).

The presence of stereocomplex crystals, creating the physical junctions between the dextran chains, was confirmed by X-ray diffraction.[77] It was further found that the length of the oligolactate chains played a crucial role in the network formation. 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.

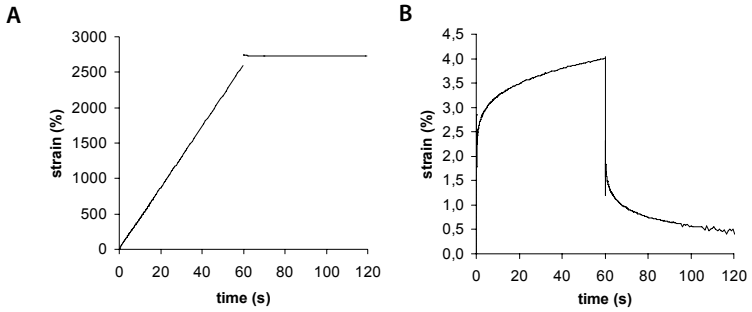

Figure 8: Creep experiments illustrating the visco-elastic behavior of a dex-L-lactate solution (A) and the elastic behavior of a mixture of dex-L- and dex-D-lactate solutions (B). Reprinted from Macromolecules 33, 3680-3686 (2000) de Jong *et al.* with permission from ACS.[75]

Apparently, longer oligolactate chains are required to result in sufficient interactions. DSC analysis showed that in blends of enantiomeric lactic acid oligomers stereocomplex formation could only occur when the DP_{av} was ≥ 7. In the case of monodisperse oligomers, coupled to dextran, the DP should at least be 11 to create hydrogels. Stronger hydrogels could be obtained by increasing the DP_{av}, the lactate substitution degree and the solid content of the gels (*i.e.* the initial polymer fraction in the gel).[78] Along with a higher network density, stronger hydrogels and longer degradation times are related. Degradation of the gels is caused by OH^- driven hydrolysis and could be 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. Longer degradation times up to approximately 60 days could be obtained with stereocomplexed hydrogels in which the dextran backbone was replaced by 2-hydroxypropyl methacrylamide (HPMA).[79] In these hydrogels, the oligolactate side chains were acetylated, preventing rapid chain end scission (backbiting) making slow random chain scission the main action of degradation. A diffusion-controlled release from dex-lactate hydrogels of the model protein 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. Low polydisperse lactate grafts, *i.e.* DP 11-14 instead of DP 1-30, led to denser network structures, as evidenced by rheology, and resulted in a slightly retarded release of both proteins.[80]

In a succeeding study by Bos *et al.* the dex-lactate hydrogels were used as *in situ* gelling system for the release of the therapeutic protein recombinant human interleukin-2 (rhIL-2).[28] From hydrogels containing 82 % water a rapid *in vitro* release was followed by a more gradual release the next days, with 65 % of the protein released within 3 days. An *in vivo* release study was conducted in SL2 lymphosarcoma-bearing mice. RhIL-2 is a broad acting T cell-derived cytokine with anti-tumor activity after local administration. 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 %. Since the tumor had infiltrated the abdominal muscles and metastasized in lungs, liver and other organs by the day the hydrogels were injected, it is noteworthy that the local rhIL-2 treatment led to a systemic effect. At day 60 of the study, all cured

mice were rechallenged with SL2 cells and appeared to be immune to the tumor. It was concluded that these stereocomplexed dex-lactate hydrogels enhance the clinical applicability of rhIL-2 therapy as it was shown 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. Bos *et al.* also reported on the *in vivo* biocompatibility and tissue reactions of the dex-lactate gels after subcutaneous implantation in rats.[81] At the outset of the study, several sterilization techniques were exploited and their influence on the network properties of the gels was investigated. Dry heat sterilization was the preferred method, since it neither degrades the lactate side chains (caused by autoclaving) nor the dextran backbone (caused by gamma irradiation). After subcutaneous implantation of the hydrogels the rats were sacrificed at various time points and the surrounding tissue was examined. It was found that at day 1 polymorph nuclear cells (PMN) had infiltrated near the gels. By day 3, PMN were no longer present, indicating that possible cytotoxic or complement activating substances were not released from or present in the gels. After 5 days, gel particles were actively phagocytosed by macrophages. By day 15 and 30, the gel had disappeared and was replaced by connective tissue. These results demonstrate 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 immune system was hardly triggered by the gel or degradation product, as evidenced by the low number of lymphocytes.

3.3.4. Ionically crosslinked dextran-based hydrogels

Nanoparticles composed of negatively charged dextran-sulfate ionically crosslinked with positively charged chitosan were described by Sarmento *et al.*[82] Complex coacervation occurred after dropwise addition of chitosan solution of pH 5.0 to dextran-sulfate solution of pH 3.2, resulting in spherical 500 nm-sized particles with a smooth surface. To obtain insulin-loaded particles, the protein was dissolved in the dextran-sulfate solution prior to chitosan addition, leading to an association efficiency of 85 %. Insulin release from these nanoparticles showed to be pH dependent. At pH 1.2, 4.5 and 5.2 no insulin was released, attributable to the overall positive charge of insulin at pH values lower than its pI (5.3), retaining the protein at the negatively charged dextran-sulfate sites. At pH 6.8 sustained insulin release was observed with 40 % released after 15 min followed by a slower release up to 70 % after 5 h. Sarmento *et al.* states that these release profiles suggest a dissociation-driven, pH-dependent release mechanism. This was further illustrated by a slower release from particles with a higher dextran-sulfate/chitosan ratio. Increasing the ratio from 1:1 to 2:1 decreased the total insulin release after 24 h from 76 to 59 %. Both ELISA and HPLC analysis showed that the released insulin was intact, indicative of a protein-friendly nanoparticle preparation method.

Another approach to design ionically crosslinked hydrogels, based on ionic interactions between oppositely charged dextran microspheres is reported in this thesis.

4. Expert Commentary

A large variety of polymeric hydrogels, both of synthetic and natural origin, have been developed and used for drug delivery purposes. Many of them are based on non-biodegradable polymers requiring surgical removal of the device when drug release is completed. Consequently, interest has grown in polymers that are biodegraded into harmless products. Their degradation rate should be tailorable to meet the requirements of specific applications in drug delivery and tissue engineering. Biocompatibility is another feature of candidate polymers that is of the utmost importance. As discussed in this review, dextran possesses both favorable characteristics, biodegradability and biocompatibility. Numerous approaches have been exploited for the design of dextran-based hydrogels. The major part of them involves chemical crosslinking of derivatized dextran during which encapsulated cells or proteins might be adversely affected. On the other hand, chemical crosslinking results in matrices of high mechanical strength with reproducible properties. Strategies are available to create stimuli-responsive hydrogels that react upon changes in temperature, pH, ionic strength and even glucose concentration. In the field of the physically crosslinked hydrogels composed of dextran derivatives, many paths are not yet tread on. Most promising are those strategies based on supramolecular self-assembly, leading to injectable matrices that gellify at the site of injection. Ideally, hydrogels will be designed that can act both as scaffolds to support cell growth and as delivery devices to release proteins over a controlled period of time to assist in the formation of new tissue. Giving the excellent performance of dextran hydrogels as protein delivery systems and tissue engineering scaffolds, as pointed out in this review, in the future various clinical applications can be foreseen.

5. Five-year view

Extensive research has been done on dextran hydrogels designed for biomedical applications. The various approaches, discussed throughout the text, have been listed in Table 2. Strikingly, only few of them have been investigated on their behavior *in vivo*. Furthermore, in those cases where protein delivery is envisioned, mainly the *in vitro* release of model proteins has been monitored. It is clear that research focus should shift to a more in depth application-driven investigation of the dextran hydrogels developed up till now. Special attention should be given to their performance as carriers of pharmaceutically relevant proteins and the *in vitro-in vivo* correlation should be thoroughly addressed. *In vivo* biocompatibility of the devices is of the utmost importance and has for many of the current dextran gels not been sufficiently tackled thus far. Although dextran is a biocompatible polymer, dextran derivatives or substances used for the preparation of the devices can influence their *in vivo* faith.

It can be anticipated that most of the hydrogel systems discussed in this review will be further exploited on their potentials in pre-clinical evaluations as well as in clinical trials. Likely, five years from now, a number of dextran-based hydrogels will have entered clinical trials with protein delivery as major application. The favorable properties of dextran hydrogels as tissue engineering scaffolds have also been summarized in this review. Chances are high that clinical evaluation will follow after the successful introduction of protein releasing hydrogels.

6. Key issues

- Recombinant DNA technology has lead to a whole new generation of protein-based pharmaceuticals. Since traditional pharmaceutical dosage forms (tablet, capsule) are not suitable to formulate proteins, there is a need for applicable delivery systems.
- Delivery systems should release the entrapped protein in a controlled manner, with full preservation of its bioactivity. Biodegradability and biocompatibility of the release device should be ensured.
- Hydrogels exhibit favorable characteristics as protein delivery matrices, such as high water content, tailorable network properties (and as a consequence controllable release profiles) and degradation behavior.
- Dextran is a non-toxic and highly water-soluble polysaccharide that has been clinically used for over 50 years as plasma volume expander. The hydroxyl groups along the dextran backbone render it particularly suitable for derivatization and subsequent crosslinking to yield hydrogels.
- In recent years, there is a growing interest in self-assembling hydrogels that are injectable and gellify *in situ*.
- The soft and rubbery nature of hydrogels and the low tendency of cells to adhere to the hydrogel surface certify minimal tissue irritation and make them attractive candidates as tissue engineering scaffolds.

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