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2. Dextran-based materials for biomedical applications

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Abstract. Dextran is a bacterial polysaccharide consisting essentially of α-1,6 linked glucopyranoside residues with a small percentage of α-1,3 linked residues. In the first part of the chapter we discuss methodologies to chemically modify dextran. Taking into account our previous work, we focus our attention in the oxidation of dextran and in the transesterification of dextran by enzymatic means. We discuss reaction mechanisms, strategies to control the degree of oxidation and acylation, the effect of oxidation and acylation in the final molecular weight of the derivatives, and methodologies to assess the degree of oxidation and acylation. In the second part of the chapter we discuss the use of oxidized or acylated dextran in the preparation of dextran-based hydrogels. Gelation times, swelling properties, mechanical properties, degradation profiles, cytotoxicity and *in vivo* profiles are given. In the third part of the chapter we focus our attention in the biomedical applications of dextran-based hydrogels namely as drug delivery systems, as scaffolds for tissue engineering, and as antifungal materials. Furthermore, we discuss strategies to design hydrogels, based in the oxidized and acylated dextran, that respond

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to external stimuli, strategies to load the drugs within hydrogels, strategies to make hydrogels cell-adhesive and to immobilize antifungal compounds. Finally, in the last section of the chapter, future directions are discussed in the use of dextran as a building block in the biomedical area.

1. Dextran and its importance in the biomedical field

Dextran is a bacterial polysaccharide, consisting essentially of α-1,6 linked D-glucopyranose residues with a few percent of α-1,2, α-1,3, or α-1,4-linked side chains (**Fig. 1A**). Dextran is widely used for biomedical applications due to its biocompatibility, low toxicity, relatively low cost, and simple modification. This polysaccharide has been used clinically for more than five decades as a plasma volume expander, peripheral flow enhancer, antithrombolytic agent and for the rheological improvement of, for instance, artificial tears (1).

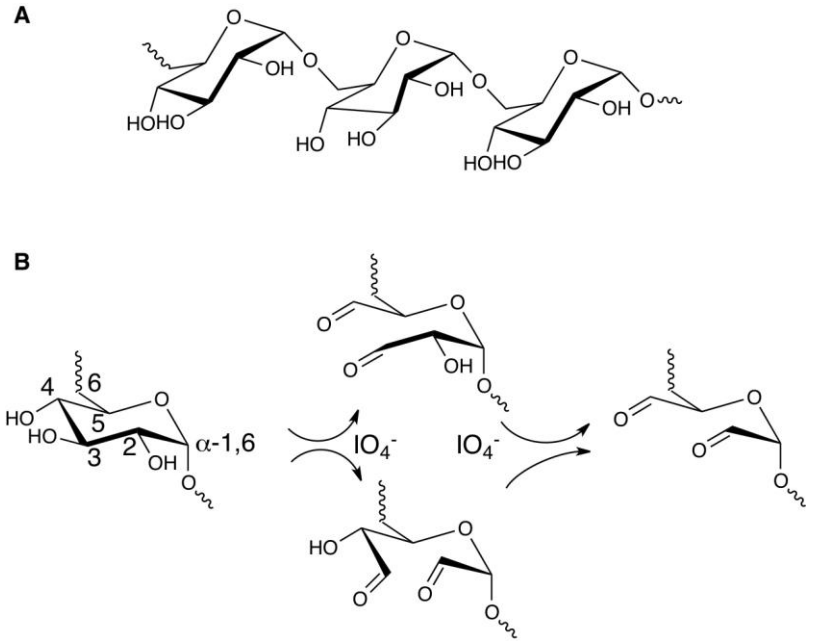

Figure 1. Structure and oxidation reaction of dextran. (A) Chemical structure of dextran. (B) Oxidation reaction of dextran with sodium periodate. The first periodate ion attacks either the C_3-C_4 or the C_3-C_2 before the second attack yielding a doubly oxidized residue.

Dextran is highly water-soluble and very stable under mild acidic and basic conditions. It contains a large amount of hydroxylic groups which turns it suitable for derivatization and subsequent chemical and physical crosslinking (2). Dextran is chosen in many biomedical applications because it is slowly degraded by human enzymes as compared to other polysaccharides (e.g. glycogen with α-1,4 linkages) and cleaved by microbial dextranases in the gastrointestinal tract. Furthermore, it has been used as macromolecular carrier for delivery of drugs and proteins, primarily to increase the longevity of therapeutic agents in systemic circulation. Dextrans have been used to decrease the *in vivo* immunogenicity of proteins and enzymes. Molecules with M_w< 40 kDa can be eliminated through renal clearance and have a half-life of 8 h, whereas molecules with M_w > 40 kDa have larger half-lives and would be sequestered in the liver and spleen and then hydrolysed by endo and exodextranases (1, 3).

Nowadays the applications and the use of dextran go far beyond its initial use as a molecule-carrier or drug delivery system. It has found applications in nanoscience, which enlarged its practical use and value. Recently, it has been used in advanced *in vivo* imaging techniques such as diagnostic magnetic resonance (DMR), magnetic resonance imaging (MRI), positron emission tomography (PET) imaging, and finally, in photodynamic therapy (PDT) that may lead to promising treatments in atherosclerosis and cancer (4).

2. Chemical modification of dextran

The high density of hydroxyl groups in dextran makes possible different kinds of chemical modifications (5). In this chapter we will focus our attention to the chemical modification of dextran by oxidation and by enzymatic means based on our previous experience (6, 7). The chemical modification of dextran by other strategies has been covered in previous reviews published elsewhere (1, 2, 5).

2.1. Oxidation of dextran

Dextran oxidation by periodate ion is a catalysis-free aqueous reaction which yields a purified product with a simple dialysis step, followed by freeze-drying. Initially described by Malaprade in 1928 (8), this method was used for the characterization and elucidation of the polysaccharide structure, through the complete oxidation and consequent analysis of the degradation products (9-12). Dextran's glucose residue has vicinal diols, presenting two different oxidisable bonds, within the same residue. The oxidation of any of those bonds yields an aldehyde on C_3, which is also susceptible to periodate

oxidation because of the presence of hydroxyl groups in C_2 or C_4, eventually leading to a double oxidation of that same residue (**Fig. 1B**). This process may be monitored by a drop in pH, due to the release of the C_3 as formic acid (6). In the first oxidation step, one of the I-O bonds of the periodate ion, attacks one of the two hydroxyl groups of the vicinal diol; the second step is the formation of the planar cyclic ester as part of an octahedral intermediate, the rate of which, must depend on the acidity of the oxygen of the OH groups and their relative positions (13). Regarding the first oxidation step, this oxidative attack, on aqueous environment, is sensitive to the carbon bond. The cleavage of the C_3-C_4 bond was described to be favored 7.5-fold compared to the C_2-C_3 bond. However, the second oxidation step was reported to have similar kinetics (14).

When the oxidation takes place in dimethyl sulfoxide (DMSO), a non-selective, single oxidation is typically observed (15). The inhibition of the second oxidation is likely due to a protective intra-residual hemiacetal formation, which does not allow further oxidation on the same residue (15). This protective effect has been also suggested to occur in aqueous environment, but it is not stable enough to prevent complete dextran oxidation (14). The hemiacetals, as well as hemialdals, should be responsible for the spectroscopic disappearance of the aldehyde moiety (6). These structures exist in equilibrium, allowing the aldehydes to be reactive towards their natural counterparts (14). Another set of studies, focused on the spectral characteristics of the mildly oxidized dextrans at different pH conditions, suggested alternative structures to hemiacetals. The UV analysis, disclosed the presence of bands, indicating that the hemiacetals might exist in a restricted pH window, between 4.0 and 5.2. Below pH 4, the bands were attributed to aldehydes and above pH 5.2, to enols and enolate ions. Drobchenko and colleagues propose a pH-dependent aldo-enol tautomerism (16). The ^{1}H-NMR analysis of dexOx at different pH's revealed the appearance of new peaks (7.5 – 9.5 ppm), which disappeared after treatment with a borohydride salt, therefore being attributed to the aldo-enol transition populations and hemiacetals (17-19). However, when the dexOx oxidation reaction is followed by ^{1}H-NMR a putative aldehyde at ~9.7 ppm is observed. At that moment, the pH is close to 2.5, but the following spectra failed to capture the aldehydes again (6).

The periodate oxidation of dextran is a harsh reaction that leads to chain degradation and alters dextran physico-chemical properties. Oxidized dextran is less soluble in water and higher viscous than regular dextran. The high viscosity could impair injectability and mixibility of certain formulations (7, 20). The degree of oxidation (DO) is also reflected on the glass transition temperature according to dynamic mechanical thermal analysis. Interestingly,

the hemiacetals do not seem to affect the thermal properties of the freeze-dried samples. The glass transition temperatures shift is in direct accordance with the molecular weight decrease, but do not give much insight on the structural consequences of the oxidation. On the other hand, the differential thermogravimetric profiles, for the diverse samples, are quite interesting. An increase in the thermal decomposition complexity with the increase in the oxidation degree was observed, evidencing the deleterious effect of periodate oxidation on dextran's structure. Low oxidative conditions, up to 10%, do not damage extensively dextran's structure, however milder oxidation conditions, result in extensive disruption of the dextran structure. These set of results may rise awareness for the consequences of periodate oxidation on the final structural properties of the modified dextran, helping researchers to better guide the design of dexOx-based formulations.

The control of DO in dextran is of utmost importance for its final properties and applications. As the DO can deeply affect the final characteristics of this macromonomer, its correct determination will be fundamental for the efficient design of hydrogels (*21-23*), nanoparticles (*24*), surface coating (*25*) and immobilization of drugs (*26, 27*). The aldehyde groups generated can be easily titrated and quantified either by colorimetry or by potentiometry. The most common methods described in the literature involve the titration with carbazate and its indirect quantification of the excess with trinitrobenzenosulfonic acid (TNBS) (*6, 28*). The dinitrosalycilic acid method, developed for estimating sugars in urine (*29*), may also be used to quantify the DO of oxidized dextran (*30*). Potentiometric titration may also be used to quantify the released equivalents of HCl, after hydroxylamine hydrochloride reaction with the aldehydes (*31*). In addition, the complex between aldehydes and fuchsin (absorption at 556 nm) may also be used to quantify the DO (*32*). As the dextran structure is very homogenous, the ^{1}H-NMR spectrum is well resolved, when compared to other polysaccharides. The carbazone formed, after titration with slight excess of *tert*-butyl carbazate (tBC) (taking into account two aldehydes per residue), yields a new non-exchangeable proton, visible on D_2O, around 7.3 ppm (6). The ratio between this peak integral and the anomeric peak, correlated well with the data from the TNBS assay, validating this ^{1}HNMR approach. As discussed above, two carbazones per oxidised residue are expected; however, early HMQC NMR data indicates a single correlation suggesting the formation of a single carbazone per oxidized residue (6). However, a closer observation, with higher resolved bi-dimensional NMR, showed two distinct peaks around 7.3 ppm (20). These peaks were attributed to the C_3 carbons arising from mono-oxidized residues in mildly oxidized dextran (20).

2.2. Transesterification of dextran by enzymatic means

Our group was the first to report the transesterification of dextran by enzymatic means (33, 34). We demonstrated that specific enzymes have a better control in the regioselective acylation of dextran, which in turn could generate better building blocks for the preparation of organized three-dimensional hydrogels (33). In one study, we found that two enzymes were able to functionalize dextran with vinyl acrylate (VA). Although Proleather protease and *Candida rugosa* lipase were partially soluble in dimethyl-sulfoxide (DMSO), they remained catalytically active in the organic solvent for at least 12 h (34). In the presence of active Proleather, monomer conversion reached ca. 22 and 60% after 15 min and 12 h of reaction time, respectively. Similar results were obtained with *C. rugosa* lipase. In the presence of active *C. rugosa* lipase, the efficiency of the coupling reaction was lower than that obtained by Proleather, however higher than the results obtained in the absence of enzyme or in the presence of thermally deactivated enzyme (DS of 14% in 72 h).

Preparative-scale reaction for the preparation of acrylated dextran was also demonstrated. Most of the VA was attached to dextran (efficiency > 71%) and that it was possible to control the degree of substitution by varying the molar ratio of VA to dextran (34). Furthermore, reasonable yields were also obtained (> 45%) using polymer purification involving an acetone precipitation step followed by dialysis. Results obtained by uni- and bidimensional NMR showed that Proleather lead to the formation of two isomers at positions 2 and 3 in the glucopyranosyl residues of dextran. According to the ^{13}C quantitative NMR results, the positional isomer ratio in acrylated dextran with degree of substitution of 31.5% was 43:57 at positions 2 and 3, respectively. Furthermore, the NMR results showed that all the glucopyranosyl residues modified were monosubstituted. Interestingly, the substitution pattern of dextran was slightly different when *C. rugosa* lipase was used rather than Proleather. Two positional isomers at positions 2 and 3 were obtained; however, the regioisomer at position 3 was more highly favored (a ratio of 28:72 for isomers at positions 2 and 3, respectively). The results obtained seem to indicate that both enzymes could not distinguish perfectly the two secondary hydroxyl groups at 2 and 3 positions; however the substitution pattern was different from acrylated dextran obtained from chemical routes (34).

In the course of investigation on the enzymatic modification of polysaccharides in organic solvents it was found unexpectedly that some enzymes could perform a double transesterification reaction leading to the formation of a hydrogel (33). From a range of eleven enzymes, the protease from *Bacillus subtilis* (Proleather FG-F) and the lipases from *Candida rugosa*

and *Pseudomonas cepacia* (Lipase AY and PS), were able to catalyze the transesterification of dextran (M_w 70,000 Da) with divinyl adipate (DVA) yielding a gel product. The conversions obtained after 48 h with Proleather FG-F, lipases AY and PS were 71.4%, 62.6% and 58.4%, respectively. NMR experiments showed that the DVA molecules added to the reaction were attached to dextran backbone at positions 2, 3 and 4 (due to the overlapping of the dextran bands the ratio of the positional isomers could not be determined). This differs from our previous results in the enzymatic transesterification of dextran with VA where hydroxyls at positions 2 and 3 were the only attachment points. Therefore, this highlights that the nature of acylating agent plays an important role in the enzyme regioselectivity.

3. Type of dextran-based hydrogels according to their preparation

Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids (35). These networks have been used as membranes for wound dressings (*23*), as membranes to prevent peritoneal adhesions (36), delivery systems for gene therapy and protein controlled-released systems (1), tissue adhesives (37), scaffolds for tissue engineering (38), antimicrobial matrices (39, 40), among others.

3.1. Dextran-based hydrogels obtained by chemical means

Several dextran-based hydrogels have been described in the literature either chemically or physically crosslinked. A very comprehensive review on cross-linking methods of hydrogels can be found elsewhere (41, 42), as well as on biodegradable dextran hydrogels (*2*). Hydrogels chemically crosslinked have been obtained either in a single step using bifunctional crosslinking agents, or in two steps involving the derivatization of dextran with polymerizable double bonds followed by free-radical or UV polymerization of the dextran derivatives. In general, the swelling of dextran-based hydrogels decreased as the degree of substitution (DS) and the concentration of macromers in the reaction mixture increased (43-45). An exception to this pattern was displayed by dextran-maleic acid hydrogels (45). In this case, the swelling increased as the DS increased. This was explained by the increase of electrostatic repulsions among ionized carboxyl groups at neutral pH´s.

The hydrophilic nature and reactivity of oxidized dextran has been exploited in the synthesis of hydrogels either as main chain or as crosslinker. The aldehyde groups may react with amines, hydrazides and hydroxyl

groups, without the need for chemical initiators or other adjuvants. Other initiator-free crosslinking methods include the pairs thiol/acrylate (46) or hydroxyl/isocyanate (47); however, in the last case, the crosslinking reaction occurs in aprotic solvents, because of the rapid reaction of water towards the isocyanate group. Gelatin was one of the first materials to be crosslinked with dexOx in order to yield a hydrogel (48). This initial study identified a set of conditions to decrease the gelation time by the increase of DO and dextran molecular weight. The onset of the reaction can be controlled by the reaction pH, ionic strength and temperature (48, 49). The hydrogel was evaluated as a drug delivery system, particularly, for the release of bovine serum albumin (BSA) and epidermal growth factor, (EGF). It was shown that the protein molecular weight and the lysine content influenced the release profiles. As this system is susceptible to interact with proteins, it retained BSA much easier (68 kDa; 60 Lys residues) than EGF (6 kDa; 2 Lys residues) (*22*). *In vitro* cytotoxic studies and *in vivo* subcutaneous implantation studies show that the hydrogel has acceptable biocompatibility as compared to commercially available wound dressing materials (*23*).

Hydrogels of carboxyethyl chitosan (CEC) crosslinked with dexOx have been also reported (50). The hydrogels of dexOx and CEC have fast gelation rates (30 – 600 seconds), depending on the polymers concentration, feed ratio and mixing temperature and could be modulated to closely match the mechanical properties of the tissue of interest. The hydrogel had a highly porous interior structure with both their swelling behavior and degradability dependent upon the relative contents of dexOx and CEC (51). The results of *in vitro* cell culture studies reveal that the hydrogel is non-cytotoxic and biodegradable. Furthermore, dexOx/CEC hydrogels administered to mice full-thickness transcutaneous wound models demonstrates good efficacy of accelerating wound healing (51).

Hydrogels obtained by the crosslinking of dexOx with adipic acid dihydrazide (AAD) have been reported by us (**Fig. 2**) (6). By controlling the DO in dextran and the initial concentrations of dexOx and AAD in the reaction mixture we could tune the gelation rate, mechanical properties, porosity, swelling ratios and dissolution profiles of the hydrogels (6). The DO of dexOx affects its viscosity and limits the concentration in the hydrogel formulation. Low oxidized samples can be used up to 30 % (w/w) while highly oxidized samples can be used up to 15% (w/w) (7). In our studies, the gelation point was evaluated by rheology and defined when the elastic modulus was equal to the loss modulus. For mild and highly oxidized samples, the gelation period decreased with increasing AAD concentration. In contrast, for low oxidized samples, the gelation period increased with increasing AAD concentration. In this last case, the excess of AAD increased

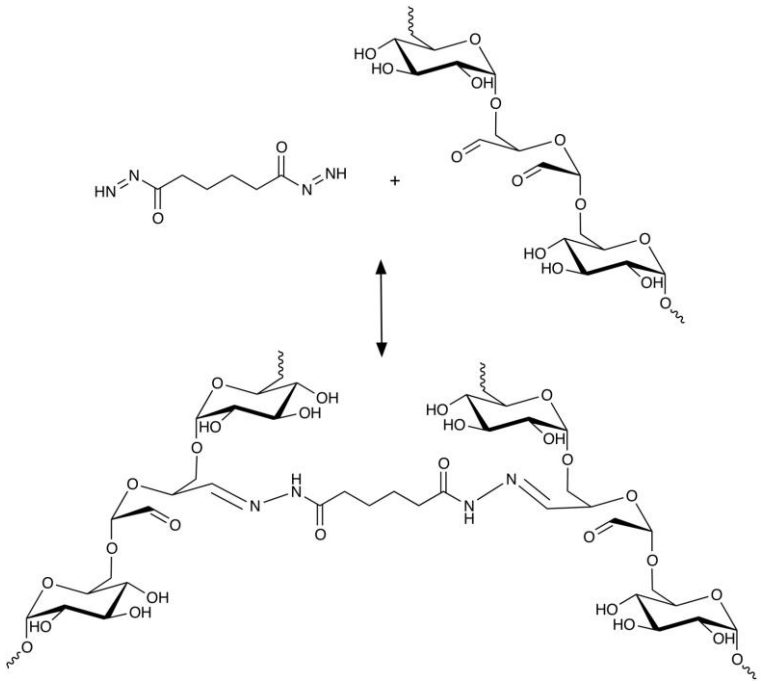

Figure 2. Crosslinking of oxidized dextran with adipic acid dihydrazide leading to the formation of hydrazones.

the number of dangling ends (52) and retarded hydrogel formation (7). The DO of dexOx and concentration of AAD are very important to control the degradation profile of hydrogels. The hydrazone bond is labile at physiological pH yielding hydrogels with variable swelling ratios and dissolution profiles. The degradation profile of hydrogels can be tailored from hours to months by controlling the initial concentration of AAD and the DO of dexOx (7).

3.2. Dextran-based hydrogels obtained by chemo-enzymatic or enzymatic means

We were one of the first groups to report the chemo-enzymatic and enzymatic preparation of dextran hydrogels (33). The reason for the use of enzymes for the modification of dextran was due to their exquisite regioselectivity. Regioselectivity may be necessary to provide highly ordered,

swellable and strong hydrogels (53). We have developed two different strategies to obtained dextran-based hydrogels using enzymatic means. In the first one, dextran was modified enzymatically by an activated ester and then crosslinked by chemical means (34). Initially, dextran was enzymatically derivatized with vinyl acrylate. Then, aqueous solutions of dextran acrylate were converted into hydrogels upon free radical polymerization. Hydrogels with different equilibrium swelling ratios and physical properties were obtained (34). These dextran-based hydrogels may have special use as drug delivery matrices for colonic targeting, particularly because of their expected degradation by dextranases (which are known to be present in the colon (*54*)) and as implantable peptide/protein delivery systems. The biocompatibility of these hydrogels has been evaluated *in vitro,* using human foreskin fibroblasts, and *in vivo,* by subcutaneous and intramuscular implantation in *Wistar* rats for up to 40 days (*55*). *In vitro* tests showed that hydrogel extracts only minimally reduced (<10%) the mitochondrial metabolic activity of fibroblasts. Moreover, cell-material interaction studies showed that these hydrogels were nonadhesive. Finally, histologic evaluation of tissue response to subcutaneous and intramuscular implants showed acceptable levels of biocompatibility, as characterized by a normal cellular response and the absence of necrosis of the surrounding tissues of the implant (*55*). In the first 10 days, the foreign body reaction in the intramuscular implantation was more severe than in subcutaneous implantation, becoming identical after 30 days. In both cases, dextran hydrogels did not show signs of degradation 6 weeks post-implantation and were surrounded by a thin fibrous capsule and some macrophages and giant cells. This response is typical with a number of non-degradable biocompatible materials.

In the second one, dextran was modified and crosslinked using enzymatic catalysis in the same step (33). These unique macroporous and ordered dextran-based biocatalytic hydrogels were prepared on a one-pot single-step transesterification reaction between dextran and divinyladipate (DVA), in neat DMSO (**Fig. 3**). Interestingly, the biocatalytic hydrogels were mechanically stronger than the chemical ones suggesting that Proleather catalysis favors the formation of a greater number of intermolecular crosslinks as compared to the chemical route, catalyzed by 4-dimethylaminopyridine (4-DMAP) (33). The structural organization of biocatalytic dextran-DVA hydrogels was also observed to be distinct from chemically synthesized dextran-based hydrogels. The enzymatic approach enabled the formation of macroporous hydrogels, with average pore diameters ranging from 34 to 0.4 μm and porosities above 80%. The homogeneity in dextran-DVA hydrogels obtained enzymatically may be at least partly due to the regioselectivity achieved in the enzymatic process (53)

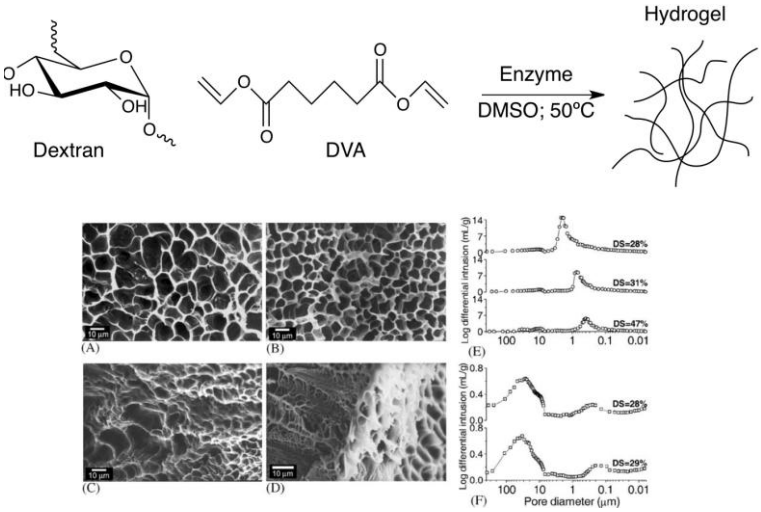

Figure 3. Proleather-catalyzed acylation of dextran with divinyl adipate (DVA). Gel characterization by scanning electron microscopy (SEM) and mercury intrusion porosimetry. (A–D) SEM from the surface of swollen dextran-DVA hydrogels in 10 mM citrate-phosphate pH 5.0, after being previously dried: DS 28% (A), 30% (B), 27% (C), 29% (D). Hydrogels were obtained either enzymatically (A,B) or chemically (C,D). (E,F) Plot of pore size distribution (log differential intrusion) against diameter of dextran-DVA hydrogels obtained enzymatically (○) or chemically (□). Adapted from reference 34.

and the uniform crosslinking of the dextran promoted by a homogeneous distribution of the biological catalyst. The elastic moduli of these hydrogels were measured by an indentation method and ranged from ca. 1.4 to 5.8 kPa when the molar ratio of DVA to dextran glucopyranose residues changed from 20 to 100%. In addition, the hydrogels were degraded under physiological conditions, likely due to the hydrolysis of the ester linkage of the crosslink molecules, in a time frame of 5 to over 40 days (33). Finally, either *in vitro* or *in vivo* studies have shown that the hydrogels were biocompatible, degradable and favored fibroblast-adhesion.

4. Applications of dextran-based hydrogels

4.1. Dextran-based hydrogels as drug delivery systems

The field of drug delivery aims at delivering any drug, with the correct timing, in a safe and reproducible manner, to a specific target in a given

concentration level. Due to their particular physical properties, hydrogels have been extensively explored in drug delivery applications. In fact, the advantage of hydrogels as drug delivery tools may be largely pharmacokinetic. Using the hydrogel as a carrier, creates a drug depot from which drugs slowly elute, maintaining a high local concentration of drug in the surrounding tissues over an extended period of time (*56*). Most of the studies used model proteins (bovine serum albumin, lysozyme, immunoglobulins) to test the efficiency of the hydrogel as drug delivery systems. However, in a few cases the drug delivery systems had biomedical significance, including the release of insulin (*57*), the release of bioactive molecules when hydrogels were used as wound dressings (*22, 23*), the release of pro-angiogenic growth factors including VEGF (*58, 59*) and the release of antimicrobial agents (*40, 60*).

The drug may be loaded into the hydrogel during its preparation (i.e. crosslinking) or after preparation by immersing the hydrogel in a solution containing the drug. The first approach is more reliable because it guarantees a known amount of drug, while the latter requires the indirect estimation of the encapsulated drug (*61*). Typically, the drug elution to the surrounding media is performed by diffusion and its rate is mainly dependent on the porosity of the hydrogel (*62*) and/or evolves with the swelling index and posterior dissolution of the hydrogel (*63*). Furthermore, the release profile is dependent on the physico-chemical characteristics of the drug, such as hydrophilicity or hydrophobicity, chemical charge or molecular size.

The dextran-based hydrogel may contain moieties that respond to external stimuli. For example, dextran-based hydrogels with thermoresponsive properties have been recently reported (*64*). The hydrogels were prepared by free radical polymerization of *N*-isopropylacrylamide (NIPAAm) monomer and a dextran macromer containing multiple hydrolytically degradable oligolactate–2-hydroxyethyl methacrylate units. Two hydrophilic model drugs, methylene blue and bovine serum albumin, were loaded into the hydrogels during the preparation of the hydrogels. The molecular size of the drugs, the hydrophilicity and degradation of the hydrogels, and temperature played important roles in controlling the drug release (*64*).

Dextran-based hydrogels may also contain moieties able to interact with biomolecules. For example, many protein growth factors have domains that are known to interact with extracellular matrix elements, such as heparin. Heparin is a sulphated polysaccharide with a negative charge and it is known to interact strongly with several growth factors, which have a heparin-binding domain such as VEGF (*65*) or the fibroblast growth factor (FGF) family (*66*)). Therefore, functionalizing dextran with carboxylate, benzylamide and

sulfate groups, yield a hydrogel-forming macromonomer, with a substantial increase on the negative charge, promoting higher retention rates of transforming growth factor beta (TGFß1) (*67*). In addition, the aldehyde moieties generated after dextran oxidation may be used to immobilize many drugs to a single polymer chain. This principle has been used to immobilize an antifungal agent such as amphotericin B (40).

4.2. Dextran-based hydrogels for tissue engineering

Tissue engineering is a research field that applies principles of biology and engineering to the development of functional substitutes for damaged tissues (*68*). Different strategies can be followed but typically when hydrogels are used they will function as scaffolds that will help guide the cells before they create their own extracellular matrix (ECM). The scaffold should provide initial mechanical support and supply biophysical and biochemical cues to sustain cell activity such as survival, proliferation, differentiation and migration. Hydrogels, which are highly hydrated crosslinked polymer networks, are thought to resemble native tissues and are thus increasingly used as cell carriers in three-dimensional (3D) tissue engineering such as the case of dextran (*69*).

Three dimensional hydrogel scaffolds incorporating a compendium of bioactive molecules within the matrix may allow a better spatial control of stem cell differentiation and ultimately the direct use of these cells for *in vivo* conditions. Enhanced vasculogenesis was demonstrated when undifferentiated human embryonic stem cells (hESC) aggregates were encapsulated for 10 days in a dextran-based hydrogel (**Fig. 4**) (38). The gels were modified with fibronectin-derived RGD ligands since fibronectin is the earliest and most abundantly expressed ECM molecule during embryonic vasculogenesis (*70*). In addition to the cell adhesion ligand, soluble factors were incorporated into the dextran matrix, using VEGF-loaded PLGA microparticles. Remarkably, the fraction of encapsulated cells expressing VEGF receptor KDR/Flk-1, a vascular marker, was increased ~ 20-fold as compared to spontaneously differentiated embryoid bodies (EB) (38). The incorporation of 0.5 mM RGD but not 5 mM RGD in the hydrogel network reduced the expression of KDR/Flk-1 marker as compared to cells encapsulated in the hydrogel without this epitope (38). Unexpectedly, the incorporation of VEGF-loaded microparticles within the dextran-based hydrogel did not increase the expression of KDR/Flk-1 marker in the hESC aggregates in a statistically significant manner for any concentration of VEGF released. Increased vasculogenesis in dextran-based hydrogel could be due to high levels of hypoxia in the encapsulated cells but it is still poorly understood. It is known

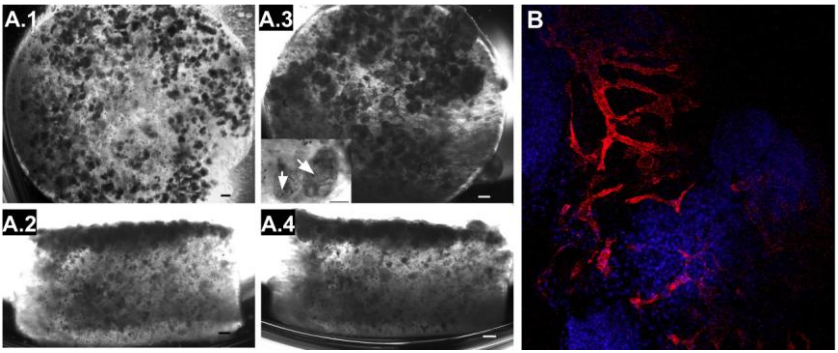

Figure 4. Bioactivity of dextran-based hydrogels in the differentiation of human embryonic stem cells. Distribution of hESC aggregates on dextran-based hydrogels with 0.5 mM Acr-PEG-RGD and 5 mg/mL VEGF-loaded 20 μm microparticles, at day 0 (A1, A2) and day 10 (A3, A4). Top (A1, A3) and side (A2, A4) views. Localization and organization of endothelial markers on hESC aggregates encapsulated in dextran-based hydrogels (B). Confocal images of $CD34^{+}$. Adapted from reference 38.

that VEGF is significantly upregulated in response to hypoxia via activation of hypoxia inducible factors (HIFs), which binds to the hypoxia-response element in the VEGF promoter (*71*).

Dextran-based hydrogels have been recently used as instructive scaffolds to promote neovascularization and skin regeneration in third-degree burn wounds (*72*). The hydrogel was based in the copolymerization of dextran-allyl isocyanate–ethylamine with polyethylene glycol diacrylate in ratio of 80/20. When the hydrogel was placed in a skin wound induced more blood flow to the burn wound area than did the control hydrogel and the wound covered with only dressing. Dextran-based hydrogels seemed to accelerate the recruitment of endothelial cells to the wound area, enabling rapid neovascularization after a week of treatment. The wound treated hydrogel resulted in skin regeneration with appendages (hair follicles and sebaceous glands).

Injectable dextran-based hydrogels are very promising for the delivery of biomolecules and cells. *In situ* crosslinkable interpenetrating double-network hydrogels composed of thiolated chitosan and oxidized dextran have been recently reported (*73*). The interpenetrating network structure was created by Schiff base formations and disulfide bond inter-cross-linkings. The cytotoxicity potential of the hydrogels was determined by an *in vitro* viability assay using fibroblast as a model cell, and the results reveal that the

hydrogels were noncytotoxic. In addition, subdermal implantation of these gels in mice models demonstrated that they were highly resistant to degradation and induced very mild tissue response.

Dextran-based hydrogels are also very useful to reduce intra-abdominal adhesions. Hydrogels formed by succinyl chitosan and dextran aldehyde significantly reduced the formation of intra-abdominal adhesions without adversely affecting wound healing (*74*). In addition, hydrogels formed by mixing hydrazide-modified carboxymethyldextran (CMDX-ADH) with aldehyde-modified dextran (DX-CHO) or carboxymethylcellulose (CMC-CHO), were very effective in the prevention of abdominal adhesion (*36*). CMDX-ADH and CMC-CHO showed minimal to mild cytotoxicity to mesothelial cells and macrophages *in vitro*, while DX-CHO was very cytotoxic. However, all crosslinked gels had very mild cytotoxicity. When applied in a rabbit sidewall defect-bowel abrasion model of adhesion formation, CMDX-CMC greatly reduced the formation of adhesions while CMDX-DX worsened them.

4.3. Dextran-based hydrogels as antifungal materials

Fungi are increasingly identified as major pathogens in bloodstream infections, often involving devices for *in vivo* applications. *Candida* spp. are the fourth most common cause of bloodstream infection in hospitalized patients (*75*). Up to 40% of patients with *Candida* spp. isolated from i.v. lines have fungemia, and the mortality rate of patients with catheter-related candidemia approaches 40% (*76*). Fungal sepsis is a leading cause of death in patients with indwelling vascular catheters, particularly in immunocompromised individuals. Materials with antifungal properties may provide an important method to prevent these infections. Dextran-based materials have been used as antifungal materials usually associated with antimicrobial molecules. In part of these strategies the dextran-based hydrogels act as drug delivery vehicles for antifungal molecules that prevent fungi from growing and proliferating (*77*). In many cases the antifungal agent is amphotericin B, a hydrophobic and very potent antifungal antibiotic (*78*).

Recently, we have developed a dextran-based hydrogel loaded with AmB (amphogel) as an antifungal agent (**Fig. 5**) (39). Amphogel killed fungi within 2 h of contact and could be reused for at least 53 days without losing its effectiveness against *Candida albicans*. The antifungal material was biocompatible *in vivo* and did not cause hemolysis in human blood. Amphogel inoculated with *C. albicans* and implanted in mice prevented fungal infection and also mitigated fungal biofilm formation. Its use can be

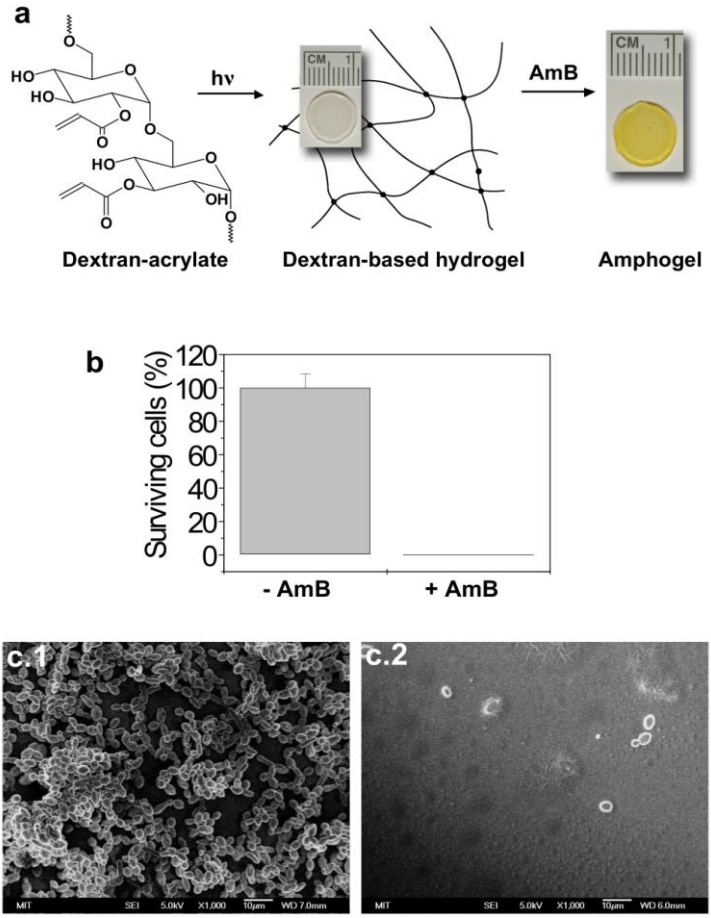

Figure 5. Antifungal properties of dextran-based hydrogels with or without AmB. (a) Schematic representation of the preparation of amphogels. (b) *C. albicans* viability on the hydrogel surface as assessed by a colony growth assay after a 2-h exposure to dextran-based hydrogels with or without AmB. (c) SEM images of dextran-based gels without (c1) and with (c2) AmB incubated with C. albicans for 48 h. Published with permission from reference (39).

extended not only as antifungal matrix but to coat a variety of medical devices such as catheters as well as industrial surfaces (39).

Dextran-based hydrogels may be also used as injectable *in situ* crosslinking networks for the delivery of amphotericin (40). In this case,

AmB was firstly reacted with dexOx and the resulting conjugate crosslinked with carboxymethylcellulose-hydrazide. The gel provided *in vitro* release of antifungal activity for 11 days, and contact with the gel killed *Candida* for three weeks (40). The gel had acceptable *in vivo* biocompatibility after implantation in the murine peritoneum.

The contribution of aldehyde groups to the toxicity of polymer-drug conjugates, such as dextran-amphotericin B (AmB) have been evaluated (*79*). Modification of the imine conjugate with ethanolamine reduced its toxicity toward the RAW 264.7 cell line by 100%. Hence the aldehyde groups were modified into imine conjugates with ethanolamine and the results had direct implications toward the safety of AmB-polysaccharide conjugates used against fungal infections. This can lead to its use not only for antifungal infections but also for leishmanial infections (*79*).

5. Future directions

From a biomaterial point of view, dextran was regarded for many years as a non-bioactive polymer because it was thought that cells could not recognize it as a biological agent. However, this may be not necessarily true and many efforts are needed to understand how cells react to this polysaccharide. Although the receptors and signalling pathways are identified in case of polysaccharides such as hyaluronic acid, in case of dextran the same level of information has not been obtained so far. Yet, some pieces have already been gathered. Lectin-type receptors, especially CD205 and CD206 have been implicated in the uptake of dextran by dendritic cells. The CD205 is found on the cell surface of macrophages and a subset of endothelial cells (*80*). Furthermore, some biological effects of dextran are already known. For example, dextran interferes with the muscarinic receptor G protein coupling in the heart and it decreases the binding of the muscarinic agonist oxotremorine-M to the muscarinic receptor (*81*). In addition, dextran-based hydrogels improve wound healing although the mechanism is not yet understood (*72*). Therefore, future work should be performed in order to understand the biological role of dextran-based biomaterials.

Oxidized or acylated dextran are building blocks that may be find particular application in the field of nanotechnology and the immobilization of biomolecules. Many antibiotics and anticancer agents are very hydrophobic and thus the conjugation with water-soluble polymers may facilitate their bioavailability. For example, doxorubicin (a member of the anthracycline ring antibiotics) has been used in the treatment of solid tumours and leukemias; however, it exerts cardiotoxicity if administer at high doses. To prevent its cardiotoxicity effects doxorubicin has been conjugated to

DexOx (*82*). It has been shown that the conjugate decreases the body clearance of the drug and prolongs its circulation in the blood (*83*). Furthermore, the conjugate accumulates preferentially in the liver and not at the heart and thus reducing its toxic effects at the heart. Recently, we have used oxidized dextran to immobilize AmB, a very hydrophobic antifungal agent, on top of a silica nanoparticle (*84*). These antifungal nanoparticle conjugates were fungicidal against several strains of *Candida sp.* mainly by contact and they could be reused up to 5 cycles without losing their activity. Therefore, oxidized and acylated dextran offer several opportunities for the solubilization of hydrophobic drugs that should be explored in future work.

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