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Synthesis and characterization of new injectable and degradable dextran-based hydrogels

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Abstract

Injectable and degradable hydrogels are very interesting networks for drug delivery and cell transplantation applications since they can be administered in the human body in a minimally invasive way. In most cases, the crosslinking reaction occurs by photopolymerisation or free radical polymerisation; however, the use of chemical initiators may promote cell death. In the current work, injectable and degradable dextran-based hydrogels were prepared without the use of initiators. Dextran, a natural glucose-containing polysaccharide, was oxidized with sodium periodate (dexOx) and the derivatives characterized by NMR and FTIR spectroscopy's as well as by colorimetric techniques. The oxidized derivatives were crosslinked with adipic acid dihydrazide (AAD), forming a gel within 2–4 min. The obtained hydrogels were characterized by their mechanical properties, swelling and degradation behavior under physiologic conditions. In addition, the hydrogel interior morphology as well as porous structure was evaluated by scanning electron microscopy (SEM) and mercury intrusion porosimetry (MIP). MIP analysis showed that dexOx hydrogels crosslinked with 10% of AAD were macroporous with pore sizes ranging from 0.32 to 0.08 μm. As expected, the average pore size increased during hydrogel degradation as confirmed by SEM and MIP studies.

Keywords: Hydrogels; Degradation; Pore size

1. Introduction

The hydrophilicity of polysaccharides, along with the ease in which they can be formed into hydrogels, makes these materials ideal for many biomedical applications including systems for peptide and protein delivery and scaffolds for tissue engineering applications [1–5]. The variety of saccharide monomers (ca. 200) and the variety of possible *O*-glycoside linkages result in a diversity of polysaccharide structures, which is important in the design of hydrogels with different physicochemical properties [6]. Because polysaccharides are natural products, they are usually biocompatible and some of them are biodegradable in the human body.

From the polysaccharides found in nature, dextran has particular relevance in the biomedical field. For several years, dextran has been used as a macromolecular pro-drug and as a plasma expander [7]. These glucose-based polymers are available in a wide range of molecular weights and contain a high density of hydroxyl groups that make the polymers highly hydrophilic and capable of being further functionalized chemically. Dextran is also biocompatible [3] and can be degraded through the action of dextranases in various organs in the human body, including liver, spleen, kidney, and colon [7].

A common way of making hydrogels with polysaccharides involves the derivatization of the natural polymer with vinyl compounds followed by either radical [1,2] or UV [8] polymerisation of the polymer derivatives. However, this approach has some limitations since it requires the use of chemicals or photo initiators, which may promote cell death [3,9,10]. This is an important issue when these networks are used as subcutaneous drug delivery systems and scaffolds for tissue engineering. Recently, a novel methodology has been

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proposed elsewhere [11], which do not require the use of initiators. In that case, the guluronate content of alginates was isolated and partially oxidized and subsequently cross-linked with AAD. Additionally, it was shown that the hydrogels were able to support the adhesion and growth of primary rat calvarial osteoblast cells [12]. However, this methodology requires several steps to isolate the guluronate (which is eliminated in the human body in opposition to alginate [13]), which may reduce its general application in the biomedical field. Furthermore, no insights were given about the porous structure before and during hydrogel degradation at physiologic conditions and this is of crucial importance for the use of these networks in biomedical field. This parameter is a critical factor to control swelling [14], drug release behavior [15], biological interaction inside the body [16], as well as the diffusion of nutrients to nourish cells, when encapsulated inside of the hydrogel network [17].

In this work, we describe a methodology to prepare injectable and degradable hydrogels from oxidized dextran (dexOx) and adipic acid dihydrazide (AAD) without the use of any chemical initiator. DexOx macromonomers form almost instantaneously hydrogels in the presence of AAD, a non-toxic and non-mutagenic [18] covalent crosslinking molecule. The networks were evaluated regarding their swelling and rheological properties, as well their degradation at physiologic pH. In this last case, MIP and SEM were used to evaluate the porous structure of the networks. The results show that it is feasible to obtain dextran-based hydrogels with different degradation profiles, which may have particular relevance in the design of new drug delivery systems and scaffolds for tissue engineering.

2. Materials and methods

2.1. Materials

Dextran (from *Leuconostoc mesenteroides*; $M_w = 73,200$ Da, according to the manufacturer's specification), adipic acid dihydrazide (AAD), trinitrobenzenosulfonic acid (TNBS), phosphate buffered saline (PBS), and sodium *m*-periodate were purchased from Sigma (Sintra, Portugal). Diethyleneglycol and *tert*-butylcarbazate (tBC) were obtained from Aldrich (Sintra, Portugal). Trichloroacetic acid was obtained from Merck (Darmstadt, Germany). Dialysis tubes with a MWCO of 12–14,000 Da were purchased from Medicell International Ltd (London, UK). All the other chemicals and solvents used in this work were of the highest purity commercially available.

2.2. Nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR) and size exclusion chromatography (SEC) analysis

^{1}H and ^{13}C NMR spectra were acquired on a Varian Unity 500 NMR spectrometer (Palo Alto, CA) using a 5 mm broadband NMR probe. ^{1}H NMR spectra were recorded in D_2O (60–100 mg in 0.7 mL; pD of ca. 5.0) using a 90° pulse and a relaxation delay of 30 s. The water signal, used as reference line, was set at δ 4.75 ppm and was partially suppressed by irradiation during the relaxation delay. A total of 16 scans were added for each ^{1}H NMR spectra. Bi-dimensional ^{1}H–^{13}C heteronuclear multiple quantum coherence spectra (HMQC) were collected as a 1024×512 matrix covering sweep widths of 2500 and 11,500 Hz in the first and second dimensions, respectively, and using 64 scans/increments. Before Fourier transformation, the matrix was zero-filled to 2048×2048 and standard Gaussian weighting functions were applied in both dimensions.

FTIR spectra were recorded with a Nicolet Magna-IR 550 spectrometer (Madison, WI, USA). The dry samples were powdered, mixed with KBr, and pressed into pellets manually. The FTIR spectra were obtained by recording 128 scans between 4000 and 400 cm^{-1} with a resolution of 4 cm^{-1}.

Size exclusion chromatography was performed in a Agilent Technologies 1100 series HPLC system, equipped with a column (TSK G4000PWxl Tosoh Bioscience) and a refraction index detector (Optilab rEX) from Wyatt Technology Corporation. The injection and column system were kept at room temperature. The eluent was mili-Q water with 0.1 M $NaNO_3$ at a flow rate of 0.2 mL/min. The SEC chromatograms were obtained from samples dissolved in the eluent with a concentration of 0.5% (w/v). Calibration was made with dextran standards (Fluka Chemie AG, Switzerland) in the molecular range of 12–80 kDa.

2.3. Synthesis of oxidized dextrans

An aqueous solution of dextran (1 g; 0.125%, w/v) was oxidized with 2 mL of sodium periodate solution with concentrations ranging from 0.15 to 3.1 M (to yield theoretical oxidations from 5 to 100%), at room temperature. An equimolar amount of diethyleneglycol was added after 20 h to stop the oxidation reaction. The resulting solution was dialysed for 3 days against water, using a dialysis tube with a MWCO 12–14,000 Da, and then lyophilised (Labconco Corp., Kansas City, MO, USA). The isolated yields were ca. 75%. The scale-up of the reaction was done using the same procedure albeit using 30 g of dextran and a calculated amount of periodate to yield a theoretical oxidation of 25%.

2.4. Determination of degree of oxidation (DO)

The DO of dexOx is defined as the number of oxidized residues per 100 glucose residues (DO refers to the experimental value unless otherwise stated) and quantified by using tBC [11,19]. The carbazates react with aldehyde groups to form carbazones in the same way hydrazones are formed in the presence of hydrazides. After reacting an excess amount of tBC with the dexOx, the unreacted tBC