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Dextran and hyaluronan methacrylate based hydrogels as matrices for soft tissue reconstruction

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Abstract

Polysaccharide hydrogels have become increasingly studied as matrices in soft tissue engineering because of their known cytocompatibility. In this work cross-linkable dextran methacrylates and hyaluronan methacrylate were synthesized and their transformation into stable hydrogels was studied. The in vitro degradation behaviour of the formed hydrogels could be controlled by the polysaccharide structure and the cross-linking density. Under in vitro conditions, the formed gels had no cytotoxic effects against fibroblasts, but cells could adhere only inefficiently in long term experiments. The use of composite gels improved the adherence of cells. Different scaffold architectures were studied including porous structures and perforated gel layers.

Selected hydrogels were examined in an in vivo pilot study using a rabbit model to evaluate their biocompatibility, stability and degradation. No signs of inflammation were seen and with prolonged duration the material was degraded and lacunas were formed by immigrating or ingrowing cells. Optimizing their mechanical properties, the formed hydrogels represent promising candidates as matrices for soft tissue reconstruction.

Keywords: Hydrogels; Dextran; Hyaluronan; Methacrylates; Tissue engineering; Scaffolds

1. Introduction

Hydrogels based on synthetic or natural polymers have become of great interest as biomaterials in drug delivery devices, for the encapsulation of cells and, most recently, as matrices in tissue engineering (Hoffmann, 2002; Drury and Mooney, 2003; Elvira et al., 2005). With regard to soft tissue reconstruction, the most attractive feature of hydrogels is their capability to absorb large volumes of water matching the structural and mechanical properties of soft tissues and their extracellular matrices. A remarkable advantage of hydrogels derived from natural polymers like proteins, polypeptides or polysaccharides is their well-known biocompatibility. In many cases such materials are also biodegradable even after chemical modification. On the other hand, relevant hydrogel properties including elasticity, swelling behaviour or the rate of biodegradation needs to be carefully optimised and adapted to the desired application (Elisseeff et al., 2005).

Among the class of polysaccharides, dextran, alginic acid, chitosan and hyaluronan have been extensively studied as educts for hydrogel matrices in tissue engineering. Alginic acid is known to form ionotropic gels with divalent cations (Smidsrod and Skjak-Braek, 1990), whereas especially chemically cross-linked hydrogels (Hennink and van Nostrum, 2002) were investigated using the other polysaccharides mentioned. For example, di- or polyfunctional low-molecular weight cross-linkers like glutaraldehyde (Jameela and Jayakrishnan, 1995), hydrazides (Vercruysse et al., 1997), carbodiimides (Park et al., 2002) or divinyladipate (Ferreira et al., 2005) were employed to fabricate hydrogels. Most of the used cross-linking agents are highly toxic to cells. Therefore, much attention has to be paid to the purification of the formed products.

In another approach, polysaccharide derivatives modified with photochemically or thermally cross-linkable groups are used as educts for the hydrogel formation. Photochemically cross-linkable gel layers and blend hydrogels curable at 60 °C were generated for example from methacrylated hyaluronan (Baier Leach et al., 2003; Inukai et al., 2000). Recently, dextran methacrylate esters have been prepared by treating dextran with glycidyl methacrylate (GMA) (van Dijk-Wolthuis et al., 1995;

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Lèvesque et al., 2005). Radical polymerization of these methacrylate derivatives using water-soluble initiation systems afforded hydrogels relatively stable under physiological conditions (van Dijk-Wolthuis et al., 1997a). Although for tissue engineering applications hydrogel scaffolds with controlled biodegradability are preferred, the possibility to adjust the cross-linking density by using dextran methacrylates with varying building units and methacrylate contents is a key factor of this approach. The further tailoring of these dextran derivatives seems to be a promising way to obtain hydrogels matching the requirements of cell cultivation scaffolds.

In this context we studied the fabrication of dextran and hyaluronan methacrylate based hydrogels with adjustable biodegradation behaviour. The in vitro cytocompatibility and cell adhesion behaviour of the novel hydrogels were studied and the potential of structured composite hydrogels to act as scaffold materials in soft tissue engineering was screened in a first pilot in vivo study.

2. Materials and methods

2.1. Materials

Dextran (from *Leuconostoc* ssp., M_r = 15,000–20,000), dimethyl sulfoxide (DMSO, 99.5%, <0.005% water), glycidyl methacrylate (97%, stabilized by 0.005% hydrochinone monomethylether), 4-(*N*,*N*-dimethylamino)pyridine (DMAP, 98%), *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (EDC, purum, ≥98%), diaminoethane (puriss. p.a., absolute ≥99%) were obtained from Fluka Chemie, Buchs, Switzerland, hyaluronan (from *Streptococcus*, M_W = 1 Mio) from Aqua Biochem Dessau, Germany. Carboxymethyl dextran (CMD, DS = 1.4) was prepared as described earlier (Wagner et al., 2004).

2.2. Analytical methods

NMR spectra were recorded in D_2O (99.9%, Aldrich), with a Bruker Advance 400 MHz spectrometer. D_2O at 4.75 ppm was used as reference line. The KBr technique was employed for recording FT-IR spectra with a FT-IR-Spektrometer *FTS 175* (BIO RAD, Krefeld, Germany). Elemental analyses were measured with a CHN-automate *CHNS-932* (Leco, Mönchengladbach, Germany). Degradation studies were performed in simulated body fluid (SBF) medium at 37 °C. The weight loss or increase was monitored gravimetrically at several time intervals.

2.3. Synthesis of polysaccharide derivatives

2.3.1. General remarks

Purification of all polymers was performed by dialysis against distilled water followed by lyophilization of the solutions and drying of the resulting polymers under vacuum. Polymer yields were in the range between 75 and 85%. The degree of substitution (DS) of the dextran and hyaluronan derivatives was determined by ^{1}H NMR using D_2O as solvent according to (Lèvesque et al., 2005). In addition, for the N-containing polymers nitrogen determination by conventional elemental analysis was used to calculate the DS values.

2.3.2. Dextran methacrylate (D-MA)

The procedure of van Dijk-Wolthuis et al. (van Dijk-Wolthuis et al., 1995) was used. Briefly, in a 100 ml round-bottomed flask 1 g (6.2 mmol) of dextran was dissolved in 30 ml of DMSO with stirring under nitrogen atmosphere. After dissolution of 200 mg (1.6 mmol) of DMAP, 820 μl (6.2 mmol) of GMA were added and the reaction mixture was stirred at room temperature for 48 h. Purification afforded a dextran methacrylate with a DS of 1.0.

FT-IR (KBr, cm^{-1}): 1019, 1158, 1225, 1409, 1457, 1637, 1713, 2930, 3200–3600.

2.3.3. Aminoethyl carbamidomethyl dextran (ACMD)

One gram (3.66 mmol) of CMD (DS = 1.4) was dissolved in 50 ml of deionized distilled water, and the pH of the solution was adjusted to 4.75. The solution was cooled to 4 °C, and 0.97 g (5.1 mmol) of EDC was added. After stirring for 30 min in an ice-bath, 342 μl (5.1 mmol) of diaminoethane was added and the mixture was stirred overnight at room temperature. Purification afforded ACMD with a DS of aminoethylamide groups of 0.8.

FT-IR (KBr, cm^{-1}): 1012, 1106, 1322, 1416, 1594, 1652, 2931, 3000–3600.

2.3.4. Methacrylated CMD (MA-CMD)

One gram (3.8 mmol) of CMD (DS = 1.5) was dissolved in 50 ml of deionized distilled water. The pH of the solution was adjusted to 7, and 5.03 ml (38 mmol) of GMA were added. After constant stirring of the reaction mixture at 50 °C for 24 h, the reaction was stopped by the addition of 5 ml of a 20% (w/v) aqueous glycine solution and stirring was continued for another 30 min. After purification, a cross-linkable polymer with a DS of methacrylate groups of 0.13 was obtained.

FT-IR (KBr, cm^{-1}): 1013, 1106, 1163, 1325, 1420, 1603, 1719, 1751, 2929, 3100–3600.

2.3.5. Methacrylated ACMD (MA-ACMD)

Two grams (6.64 mmol) of ACMD (DS of aminoethylamido groups of 0.81) were dissolved in 100 ml of deionized distilled water. The pH of the solution was adjusted to 7, and 8.8 ml (66.4 mmol) GMA were added. After constant stirring the reaction mixture for 24 h at 50 °C, the reaction was blocked by the addition of 5 ml of a 20% (w/v) aqueous solution of glycine and stirring was continued for another 30 min. Purification afforded a methacrylated ACMD with a DS of methacrylate units of 0.8.

FT-IR (KBr, cm^{-1}): 1016, 1111, 1166, 1233, 1410, 1454, 1601, 1657, 1718, 1753, 2930, 3000–3600.

2.3.6. Methacrylated hyaluronan (HA-MA)

One gram (2.49 mmol) of hyaluronan was dissolved in 100 ml of deionized distilled water. The pH of the solution was adjusted to 7, and 329 μl (24.9 mmol) of GMA was added. After constant stirring for 24 h at 50 °C, the reaction was stopped by the addition of 5 ml of a 20% (w/v) aqueous glycine solution and stirring was continued for 30 min. Purification afforded a hyaluronan methacrylate with a DS of methacrylate groups of 0.7.

FT-IR (KBr, cm^{-1}): 1046, 1079, 1152, 1322, 1377, 1410, 1617, 1747, 2926, 3000–3700.

2.4. Hydrogel formation

Aqueous solutions containing the methacrylated polysaccharide (1–15% (w/w)) or mixtures of two different methacrylated polysaccharides and a suitable initiation system composed of ammonium peroxodisulfate (APD)/*N*,*N*,*N*′,*N*′-tetramethylethylene diamine (each 30 μl of 10% (w/w) aqueous solution per ml of polymer solution) or potassium peroxodisulfate (PPD)/triethanolamine (10 mg of PPD/ml of polymer solution/30 μl of triethanolamine) were placed in silicon moulds and cured for 2–5 min at room temperature to obtain compact hydrogels.

For the preparation of porous scaffolds, a conventional leaching procedure was used (Vogt et al., 2005). Crystalline sucrose was added to the aqueous solutions described above and the mixtures were immediately cured. Subsequently, sucrose was leached out in boiling water.

Perforated hydrogel layers were obtained by curing solutions of the methacrylated polysaccharides in structured silicon forms.

2.5. In vitro cell culture

Primary rabbit fibroblasts and 3T3 cells were cultured in DMEM/F12 1:1 (Biochrom, Berlin, Germany) containing 10% FCS, 50 U/ml penicillin, 50 μg/ml streptomycin at 37 °C under 5% CO_2 atmosphere. Medium was renewed every 2 days.