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Novel injectable and *in situ* cross-linkable hydrogels of dextran methacrylate and scleroglucan derivatives: Preparation and characterization

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ABSTRACT

In this paper mixtures of two biocompatible polymers, dextran methacrylate (DEX-MA) with different molecular weights and scleroglucan (Scl), in its native form and as carboxymethyl derivative (Scl-CM), were tested as injectable and *in situ* cross-linkable systems. Rheological and texture analyses were carried out to better investigate the behavior of this kind of matrices. The combination of these polymers is able to assure adequate mechanical properties, suitable for biomedical applications. In addition swelling studies and *in vitro* release studies of three different biomolecules were also carried out.

1. Introduction

Tissue engineering represents a great challenge for biomedical research because it ranges from the repair of damaged tissue to the release of bioactive molecules (Langer, 1999; Langer & Karp, 2007; Langer & Tirrell, 2004). Among the numerous systems proposed in this field hydrogels seem to have the most attractive characteristics (Baroli, 2007; Drury, 2003). Hydrogels are crosslinked, three-dimensional polymeric networks able to swell in aqueous media without dissolving their structure. Formed by water-soluble polymers, these systems show very similar properties to leaving tissue ones, and as a consequence well accepted for implantation. In particular injectable and/or *in situ* forming hydrogels represent a promising approach in many pharmaceutical and biomedical applications, because they allow treating injured patients by using minimal invasive techniques (Kretlow, Klouda, & Mikos, 2007). Moreover, injectable systems provide the ability to take the shape of the cavity where they are placed, thus filling possible irregular defects. Different synthetic and natural polymers have been employed as injectable and/or *in situ* forming hydrogels, whose preparations and utilizations have been exhaustively revised (Nair & Laurencin, 2006). Polysaccharides and their derivatives find employment as starting materials for the preparation of hydrogels for pharmaceutical purposes because of their biocompatibility, non-toxicity and not immunogenic properties (Malafaya, Silva, & Reis, 2007). In particular dextran (DEX) is one of the most widely used polysaccharide. It consists of a linear chain of (1→6) linked α-D-glucopyranosyl units with few (1→2) (1→3) (1→4) α-D-glucopyranosyl ramifications. The reaction of dextran with glycidyl methacrylate produces a derivative (DEX-MA) able to form hydrogels by UV irradiation, through crosslinking of methacrylate moieties (Coviello et al., 2007; van Dijk-Wolthuis, Kettennes-van den Bosch, van der Kerk-van Hoof, & Hennink, 1997). Despite the numerous applications proposed for DEX-MA based hydrogels (Kim & Chu, 2000; Meyvis, De Smedt, Stubbe, Hennink, & Demeester, 2001), these systems show rheological properties unsuitable for tissue engineering applications because they are very brittle. In the literature it is reported that mixtures of polymers can lead to the formation of new materials having properties completely different with respect to the starting polymers ones (Bajpai, Shukla, Bhanu, & Kankane, 2008; Goycoolea, Morris, & Gidley, 1995). Therefore in order to overcome the drawbacks of DEX-MA hydrogels, mixtures of DEX-MA and calcium alginate have been recently proposed as interpenetrating polymer system (IPN) for biomedical applications (Matricardi, Pontoriero, Coviello, Casadei, & Alhaique, 2008; Pescosolido et al., 2009). Mechanical characterization confirmed

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the possibility to obtain a system able to be injected and crosslinked *in situ*. Now we decided to study new systems composed of DEX-MA and scleroglucan, in its native form and as carboxymethyl derivative (Scl-CM). Scleroglucan is a polysaccharide consisting of a backbone of (1→3)-β-linked glucopyranosyl residues substituted with a single (1→6)-β-glucose residue every third backbone units. It is already employed in pharmaceutical field (Coviello et al., 2005). We choose to use a carboxylated derivative of scleroglucan (Scl-CM) that is able to give physical hydrogels (Casadei, Matricardi, Fabrizi, Feeney, & Paolicelli, 2007; Corrente et al., 2009). Binary mixtures of DEX-MA and Scl or Scl-CM and their hydrogels were prepared and the mechanical properties of all the systems were investigated. At the same time the possibility to deliver molecules of different steric hindrance loaded inside, was studied.

2. Materials and methods

2.1. Materials

All used reagents were of analytical grade. Scleroglucan with $M_w = 1.4 \times 10^6$ as evaluated by viscosimetric measurements in 0.01 M NaOH, was provided by Carbomer. Dextran (DEX) from *Leuconostoc* ssp. (M_w 40,000 and 500,000), 4-dimethylaminopyridine (DMAP), anhydrous dimethylsulfoxide (DMSO), theophylline (THP), myoglobin (MGB), chloroacetic acid were purchased from Fluka (Switzerland). Glycidyl methacrylate (GMA), methacrylic acid (MA), vitamin B12 (VitB12), D_2O, DMSO-d_6, DOWEX 50WX4-50 ion-exchange resin, Irgacure 2959 (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone) were purchased from Sigma–Aldrich (England). Dialysis tubes (cut-off 12,000–14,000) were purchased from Medicell International (UK).

2.2. Synthesis of dextran methacrylate (DEX-MA)

Dextran methacrylate was synthesized as already reported (van Dijk-Wolthuis et al., 1997). Briefly, to a solution of dextran (M_w 40,000, DEX_{40}, 5.0 g) in anhydrous DMSO (40 ml), DMAP (1.0 g) and GMA (1.50 g, 1 mol/3 mol of repetitive unit) were added. The solution was maintained under stirring at room temperature for 24 h. EtOH (200 ml) was added dropwise; the precipitated solid was recovered by filtration and dissolved in water (15 ml). The solution was neutralized with 0.1 M HCl and submitted to exhaustive dialysis against distilled water. After freeze-drying, the polymer was characterized by FT-IR, ^{13}C NMR and ^{1}H NMR. FT-IR spectra were recorded using KBr pellets with a PerkinElmer Paragon 1000 spectrophotometer in the range 4000–400 cm^{-1} (resolution of 1 cm^{-1}). ^{13}C NMR and ^{1}H NMR spectra were obtained in D_2O with a Bruker AC-400 instrument. The degree of derivatization (number of methacrylic groups for 100 glucopyranosyl residues, DD), calculated on the basis of the ^{1}H NMR spectrum, was 20 ± 1. DEX-MA with the same DD was obtained starting from DEX with M_w 500,000 (DEX_{500}) and applying the same procedure but adding 45 ml of anhydrous DMSO to 5.0 g of polymer.

2.3. Synthesis of carboxymethyl scleroglucan (Scl-CM)

Scleroglucan (1.0 g) was derivatized through reaction with $ClCH_2COOH$ in basic medium according to the method already described (Casadei et al., 2007). After elution through a DOWEX 50WX4-50 ion-exchange resin column previously treated with 2.0 M HCl, the freeze-dried polymer was characterized by FT-IR and submitted to potentiometer titration. The degree of derivatization (number of carboxymethyl groups for 100 repetitive units) was 80 ± 5.

2.4. Preparation of the polymeric solutions

The following binary mixtures were investigated:

- DEX_{40}-MA/Scl-CM
- DEX_{500}-MA/Scl-CM
- DEX_{500}-MA/Scl
- DEX_{500}-MA/$Scl_{0.2}$

After dissolution of Scl-CM (1.0%, w/v) or Scl (1.0%, w/v and 0.2%, w/v), DEX-MA (5.0%, w/v) with different molecular weight was added.

2.5. Hydrogels formation

All the polymeric mixtures (5 ml) were placed in Petri plates (d = 7.0 cm) previously treated with a polyethylene glycol solution (10%, w/v PEG 40,000 in phosphate buffer, pH 7.4), in order to easily remove the hydrogel after gelation (Kuijpers et al., 1999). A solution of the photoinitiator Irgacure 2959 (250 μl or 25 μl of a 20% (w/v) solution in N-methyl-pyrrolidone) was added just before the irradiation. The samples were irradiated with a UV lamp, G.R.E. 125 W Helios Italquarz for 5, 10 or 20 min. The hydrogels were analyzed after 24 h from the preparation in order to assure the complete crosslinking of the methacrylic groups.

2.6. Rheological measurements

Rheological experiments were performed with a Haake RheoStress 300 Rotational Rheometer (Germany) equipped with a Haake DC10 thermostat. Flow curves of all the polymeric solutions were obtained with a cone-plate geometry in the range of 0.01–100 Pa. Frequency sweep experiments were also performed on the same samples in the range 0.01–10 Hz in the linear viscoelastic region, assessed by preliminary stress sweep studies. Instead hydrogels (thickness of 1.0–3.0 mm) obtained after UV irradiation of the solutions, were submitted to oscillatory analyses using a serrated plate–plate geometry in the same conditions as before. All the experiments were carried out at least in triplicate at the temperature of 25.0 and 37.0 ± 0.1 °C.

2.7. Swelling studies

Swelling studies were performed on freeze-dried hydrogels in different aqueous media: HCl 0.1 M, phosphate buffer (PB, pH 7.4, ionic strength I = 0.1), bidistilled water and NaCl 0.1 M. Aliquots (30 mg) of the gel were placed in tarred 5.0 ml sintered glass filters (Ø 10 mm; porosity G3) and allowed to swell at 37.0 ± 0.5 °C after immersion of the filters in the swelling media. After 24 h the excess liquid was removed by percolation at atmospheric pressure. Then the filters were weighed.

The swelling degree (q) was expressed as:

$$q = \frac{W_s}{W_d}$$

where W_s and W_d are the weights of the swollen and dry hydrogels, respectively. Each experiment was performed in triplicate. The swelling ability was evaluated for the hydrogels DEX_{40}-MA/Scl-CM, DEX_{500}-MA/Scl-CM, DEX_{500}-MA/Scl and DEX_{500}-MA/$Scl_{0.2}$.

2.8. Determination of unreacted methacrylic groups

In order to investigate the amount of unreacted methacrylic groups present in the hydrogels after the photocrosslinking, about