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Radiation synthesis of biocompatible hydrogels of dextran methacrylate

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ABSTRACT

The aim of this work was to synthesize biocompatible dextran-based hydrogels through crosslinking initiated by ionizing radiation. A series of derivatives of dextran has been synthesized by coupling of methacrylated glycidyl to the structure of this polysaccharide, yielding dextran methacrylate (Dex-MA) of the degree of methacrylate substitution (DS) up to 1.13 as characterised by FTIR and NMR spectroscopy. Chemically crosslinked hydrogels were formed by electron-beam irradiation of Dex-MA in aqueous solution in the absence of low-molecular-weight additives such as catalysts, monomers or crosslinking agents. Crosslinking of Dex-MA in aqueous solutions of 20 g/l and above was an efficient process, the gels were formed at doses as low as 0.5 kGy (experiments conducted up to 100 kGy) and were characterised by high content of insoluble fraction (70–100%). Due to high crosslinking density the equilibrium degree of swelling of fabricated gels was controlled principally by the initial concentration of Dex-MA solution subjected to irradiation, and it was in the range of 20 to over 100 g of water absorbed by gram of gel. Cytocompatibility of hydrogels was examined using XTT assay through evaluation of the cell viability being in indirect contact with hydrogels. The results indicated that hydrogels of Dex-MA of the average DS below 1 were not cytotoxic. Altogether, our data demonstrate that irradiation of methacrylated dextran in aqueous solution is an efficient method of fabrication of biocompatible hydrogels, which applications in regeneration medicine are anticipated.

1. Introduction

Dextran is a non-toxic, hydrophilic, bacterially derived polysaccharide, mainly composed of linear α–1,6 linked D-glucopyranose residuals with a low percentage of α–1,2, α–1,3 or α–1,4 linked side chain (Dumitriu, 2005). Biomaterials based on this natural polymer are widely used for biomedical applications due to dextran biological activity, and well-documented biocompatibility and biodegradability in physiological environment (De Groot, 2001; Maia et al., 2014; Sun et al., 2011a; Sun and Mao, 2012). Its biomedical applications include plasma expander (de Jonge and Levi, 2001), drug delivery systems (Pacelli et al., 2015), hydrogels and wound dressings (Sun et al., 2011a).

Soft tissue reconstruction solutions and novel wound dressings that apply techniques of tissue engineering require biodegradable and biocompatible materials capable to form three-dimensional structures supporting cell proliferation and regenerative processes of tissues. A possible approach may involve controlled chemical modification of natural polymers such as polysaccharides and their further transformation into chemically-stable hydrogel. Hydroxyl groups present in the structure of dextran provide opportunity for its modifications. Hydrogen in these groups can be replaced by functional substituents yielding derivatives with specific, tailored characteristics, which can be further engineered to obtain various microstructured scaffolds including spheres, fibers or hydrogels for biomedical applications (Sun and Mao, 2012). In recent years, wide range of different functionalization of dextran has been accomplished, yielding materials of specific properties although with preserved biocompatible character of their parent polysaccharide (Pitarresi et al., 2003; Wang et al., 2012; Yuba et al., 2014).

Hydrogels are three-dimensional crosslinked polymeric networks able to absorb significant amount of water and/or biological fluids (Peppas and Mikos, 1986). Hydrogels can be formulated from synthetic materials, from natural polymers such as dextran, alginate or chitosan, and from combination of both synthetic and natural materials (Hoffman, 2012; Malafaya et al., 2007). Hydrogels of natural origin or with polysaccharides incorporated in the synthetic hydrogels, i.e. semi interpenetrating network, have been often used in biomedical field, mainly due to their specific biofunctionality. Chemically crosslinked dextran-based hydrogels have been manufactured and their potential applications in soft tissue engineering or as wound dressings were proposed (Sun et al., 2011b; Sun and Mao, 2012).

Hydrogels can be manufactured by various techniques. One approach involves cross-linking agents in hydrogel fabrication process,

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as for example poly(ethylene glycol) dimethacrylate is used for crosslinking of chitosan by radiation technique (Czechowska-Biskup et al., 2016). Nevertheless, involvement of a crosslinker carries significant risk since most of these agents are highly toxic and might cause undesirable response of organism. Thus, much attention has to be paid to the purification of the end-products. In another approach, the substrate polymer is chemically modified in terms of incorporation of cross-linkable groups prior to hydrogel synthesis. For example, urethane dextran derivatives were employed to fabricate photocrosslinkable tissue adhesive (Möller et al., 2007) or dextran methacrylate esters were used to design macroporous, interconnected scaffolds for tissue engineering (Lévesque et al., 2005). Radical crosslinking of methacrylated derivatives of dextran using potassium peroxydisulfate and N,N,N′,N′-tetramethylethylene-1,2-diamine (TEMED) as the initiating system resulted in biodegradable hydrogels, stable in physiological environment (van Dijk-Wolthuis et al., 1997). Alternatively, crosslinking of dextran methacrylate with UV-light can be achieved in its aqueous solutions without low molecular weight photoinitiator compounds, which are substituted by photocrosslinkable synthetic polymers, such as for instance poly(ethylene glycol)dimethacrylate (PEGDMA) (Yin et al., 2010), N,N,N′,N′-tetramethylethane-1,2-diamine (TEMED) (Hennink et al., 1996; Lévesque et al., 2005), poly(*N*-isopropylacrylamide) (PNIPAAM) (Zhang et al., 2004) or α,β-poly(N-2-hydroxyethyl)-DL-aspartamide methacrylate (PHM) (Pitarresi et al., 2007).

Employment of ionizing radiation seems to be an interesting alternative to classical chemical methods for obtaining hydrogels, especially for biomedical applications, when chemical purity of the product is the critical factor. Manufacturing hydrogels from synthetic polymers is usually accomplished by irradiation of an aqueous solution of a hydrophilic polymer or polymers, that leads to network formation. The process does not require any low-molecular-weight additives (Rosiak and Ulański, 1999). Synthesizing hydrogels of macroscopic dimensions (note that bulky items can be uniformly processed by gamma rays due to its high penetration capability) as well as nanogels is possible by radiation method (Kadlubowski, 2014). Application of ionizing radiation in order to crosslink natural-origin polymers such as polysaccharides is not straightforward. In general, irradiation of polysaccharides leads to scission of polymer chains, resulting in decreased molecular weight and no formation of crosslinked polymer networks (Czechowska-Biskup et al., 2007; Ulanski and von Sonntag, 2000). Nevertheless, available literature proves that crosslinking of polysaccharides using ionizing irradiation without any additives can be achieved. High-energy radiation of gamma rays has been mentioned for formulation of dextran-based thermo-sensitive drug delivery system (Almeida et al., 2013). Another example demonstrates radiation crosslinking of highly concentrated or dilute solutions of carboxymethylcellulose resulting in formulation of polysaccharide hydrogels (Wach et al., 2014).

Irradiation of neat dextran causes its severe degradation. It is expected that crosslinking process initiated by ionizing irradiation might be realized through functional groups incorporated into the polymer structure enabling efficient coupling of macromolecules. Thus, the aim of this study was to synthesize crosslinkable methacrylated dextran derivative (Dex-MA) with various degree of substitution (DS – the average number of methacrylate groups per single D-glucopyranose residue) and subsequently, examine behaviour of Dex-MA aqueous solutions under electron beam irradiation. Obtained hydrogels were characterised by sol-gel analysis and, since potential applications are in a biomedical field, cytotoxicity evaluation was carried out.

2. Materials and methods

2.1. Materials

Dextran (from *Leuconostoc ssp*., Mr=100,000) was purchased from Sigma Aldrich (Canada), dimethyl sulfoxide (DMSO, 99.5%, < 0.005% water) and hydrochloric acid (HCl, 36–38%) were obtained from Chempur (Poland). Glycidyl methacrylate (GMA 97%, stabilized by 0.005% hydroquinone monomethylether) was purchased from Sigma Aldrich (Japan), 4-(*N,N*-dimethylamino)pyridine (DMAP) and dialysis tubing cellulose membrane (cut-off 12,000–14,000) were obtained from Sigma Aldrich (USA). All other reagents were of analytical grade. Ultrapure water was obtained using Micropore system (TKA).

2.2. Synthesis of Dex-MA

The procedure of van Dijk-Wolthuis was used for synthesizing a series of methacrylated dextran derivatives with different degree of substitution (DS) of 0.2, 0.4, 0.8, 1.0, 1.2 and 1.5 (van Dijk-Wolthuis et al., 1995). To synthesize dextran methacrylate, 5 g of dextran was dissolved in anhydrous dimethylsulfoxide (DMSO) in a three-necked flask equipped with a dropping funnel. After dextran dissolution, 1 g of 4-(*N,N*-dimethylamino)pyridine (DMAP) was added as a catalyst. And then, calculated amount of glycidyl methacrylate (GMA) for anticipated degree of substitution was added dropwise into the polymer solution. The mixture was stirred under argon atmosphere for 48 h at room temperature. The reaction was terminated by adding an equimolar amount of HCl to neutralize the DMAP. To purify the reaction mixture, it was dialyzed against deionized water at 4 °C for at least two weeks. In order to obtain a highly purified product this process was monitored using UV–Vis spectroscopy (Perkin Elmer Lambda 40 UV–VIS spectrophotometer) till absorbance of dialysate at 208 nm (λ_{max} of DMSO and GMA) and 280 nm (λ_{max} of DMAP) was lower than 0.01. Finally, Dex-MA was lyophilized to obtain white, fluffy product.

Synthesized dextran derivatives were characterised using FTIR and NMR spectroscopy. FTIR spectra of dextran and Dex-MA incorporated into KBr were collected in a transmission mode with Nicolet Avatar 330 FTIR (USA) spectrometer with the resolution of 2 cm^{-1} and ^{1}H NMR spectra of polymers were recorded with a Bruker Avance II 700 MHz UltraShield Plus (Germany) spectrometer using D_2O as a solvent at 4.8 ppm as the reference. To determine actual degree of substitution of synthesized Dex-MA the peaks of relevant chemical shifts were integrated to calculate DS using the following equation:

$$DS = \frac{I_B}{1.04\, I_A} \quad (1)$$

where I_B is the average integrated region of the double bond protons at about 6 ppm, I_A is the integrated area of the anomeric proton of dextran at 5 ppm and the 1.04 number is a correction factor resulting from presence of the average 4% of α–1,3 linkages of dextran (van Dijk-Wolthuis et al., 1995).

2.3. Preparation and characterization of Dex-MA hydrogels

Dextran-based hydrogels were manufactured in aqueous solutions of Dex-MA through crosslinking of methacrylic groups using radiation initiation. Aqueous solutions of Dex-MA with different DS were prepared in advance to ensure complete dissolution of the polymer at concentrations of 0.5%, 1%, 2%, 3% and 5%. 1.5 mL Dex-MA solutions were saturated with argon for 30 min in glass ampoules and sealed with Parafilm. Then, the samples were irradiated by electron beam (EB) from a 6 MeV linear accelerator (ELU-6, Eksma, Russia) at 0.5 – 100 kGy at room temperature. Dose was determined using alanine film dosimeters.

Following the irradiation samples of permanent chemical hydrogels were transferred into deionized water and sol-gel analysis was carried out to determine equilibrium degree of swelling (EDS) and gel fraction (GF). Hydrogel samples were allowed to swell in water with daily water exchanges for at least one week in order to reach equilibrium and remove residuals of non-crosslinked or degraded polymer chains, i.e. the soluble part (sol). Additionally, selected hydrogel samples were autoclaved in order to confirm results obtained with water extraction.

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