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Polysucrose-based hydrogels for loading of small molecules and cell growth

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ABSTRACT

Cross-linked polysucrose hydrogels were synthesized for the first time from polysucrose grafted with methacrylic anhydride (MA) and crosslinked with ethylene glycol dimethacrylate (EGDMA). The addition of sucrose and polyethylene glycol monomethyl ether (mPEG5000) as porogens to the cross-linking reaction led to the formation of interconnected pores as well as a shift from a homogeneous non-porous to a heterogeneous porous surface. The potential of this family of hydrogels as biomaterial was assessed through the determination of the loading/release capacity of cationic and anionic dyes as model molecules and biocompatibility test with fibroblast cells. Cationic dyes showed high loading and sustained release over time attributed to the ionic interactions of the dyes with the hydrogels carrying a net negative charge. Anionic dyes on the other hand showed a rapid sinusoidal loading/release pattern. The release of the dyes was found to increase with increasing swelling capacity. NIH 3T3 fibroblast cells proliferated on hydrogels containing a porous structure and avoided the non-porous areas of the hydrogel surface.

1. Introduction

Hydrogels have the capacity to mimic human body tissue due to their water sorption capacity, that is, their ability to change from a glassy to a rubbery state upon contact with water. This behavior is mainly attributed to the presence of hydrophilic functional groups and is exploited for the entrapment of various molecules and organisms, such as growth factors, enzymes and cells [1–3]. Hydrogels made from natural polymers or biopolymers are very interesting candidates for tissue engineering or drug delivery applications due to their versatile properties that include biocompatibility, high swelling capacity, and non-toxicity and as well as their chemically-alterable structures. A lot of attention has indeed been directed towards dextran [4–7], chitosan [8–10], alginic acid or alginates [11–13] and hyaluronan hydrogels [14–16].

The derivatization of natural polysaccharides via methacrylation has led to hydrogels with varying permeability and diffusion patterns, which are important features for the loading and release of molecules [17]. Numerous studies relate to dextran hydrogels, their swelling behavior and the loading and release of proteins and smaller molecules [18–23]. Hennink and coworkers [24] investigated the ability of dextran glycidyl methacrylate hydrogels for the controlled release of proteins. Release of proteins [lysozyme (M = 14,000 g/mol), bovine serum albumin (BSA, M = 67,000 g/mol)] and immuno gamma globulin [(IgG, M = 150,000 g/mol)] from the hydrogels showed a correlation between protein size and hydrogel pore size. The equilibrium water content of the hydrogels itself depends on the degree of substitution of the dextran and is also a determining factor for the release kinetics. Lysozyme, which had a lower molar mass and smaller hydrodynamic diameter (D_o = 4.1 nm) compared to BSA (D_o = 7.2 nm) and IgG (D_o = 10.7), was released faster [24]. The release of smaller molecules, such as doxorubicin, alizarin red S and FITC-dextran, from dextran-methacrylate hydrogels was found to depend on the degree of substitution (DS), pH and molar mass of the molecules. High DS and high molar mass resulted in slow release. Furthermore, the presence of ionizable groups in doxorubicin and alizarin red S led to an increase in the release rate in acidic or alkaline media [25].

Polysucrose (PSuc), a polymer obtained from sucrose and epichlorohydrin (ECH) via polycondensation in the presence of sodium hydroxide, is commercialized under the trade name Ficoll® 70 PM. Polysucrose was recently derivatized via grafting of biodegradable polyesters to produce amphiphilic copolymers that self-assemble into nanomicelles in water [26]. Hou et al. [27] reported on the preparation of crosslinked PSuc microspheres by a two-stage polymerization, in

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which sucrose was first reacted with ECH in the presence of NaOH, followed by crosslinking with chlorobenzene to form soluble PSuc microspheres. The diameter of the microspheres ranged between 250 and 450 nm as determined by optical microscopy and SEM analyses and decreased with increasing ECH concentration. The microspheres exhibited a smooth surface and an interior porous structure. They swelled in water (equilibrium water content: 94%) and showed a high adsorption capacity for BSA (49 mg/g). The BSA adsorption capacity decreased as the size of microspheres increased [27,28]. In another paper, Hou et al. [29] reported on PSuc microspheres by inverse suspension polymerization using soluble PSuc, ECH as crosslinker and the dimethyl ether of polyethylene glycol as porogen. They obtained spherical beads with an average hydrodynamic diameter of 340 μm and equilibrium water contents as high as 92% to 97% with increasing content of porogen. The saturated adsorption capacities of these microspheres ranged from 42.6 to 98.5 mg/g [29].

Crosslinked polysucrose hydrogel microparticles, synthesized using divinyl sulfone as crosslinker via reverse micelles of sodium bis(2-ethylhexyl) sulfosuccinate, were shown to be a nutrient for *Escherichia coli* and could be used as a growth medium for other cells, bacteria and organisms [30]. The in vivo safety of these microparticles was demonstrated using MDA MB-231 cancer cells and L929 fibroblast cells.

In this paper, we report on the preparation of polysucrose-based hydrogels with or without porogens. Porogens such as sucrose or poly(ethylene glycol) were used to enhance porosity and pore size of the hydrogels. Their ability to load cationic or anionic dye molecules via the soaking method and in situ cross-linking was examined. The kinetics of dye release at physiological pH was also assessed. Finally, the capacity of the hydrogels to grow NIH3T3 fibroblast cells was investigated.

2. Materials and methods

2.1. Materials

Ficoll® PM 70 [M_w 70,000], ethylene glycol dimethacrylate 98% (contains 90–110 ppm monomethyl ether hydroquinone as inhibitor), triethylamine ≥ 99% (TEA), *N*,*N*,*N*′,*N*′-Tetramethylethylenediamine 99%, (TEMED), ammonium persulfate, *ReagentPlus*®, ≥99.99% (APS), methacrylic anhydride (2000 ppm topanol A as inhibitor), 94%, lithium chloride, anhydrous ≥ 98% (LiCl) and *N*, *N*-Dimethylformamide ≥ 99.8% (DMF) as well as the dyes Nile blue A sulfate (NBA), Brilliant green (BG), Sulforhodamine B (SRB) and Fluorescein sodium salt (FSS) were all purchased and used as received from Sigma Aldrich. Sucrose was purchased from a local supplier and mPEG5000 was bought from International Laboratory, USA. Pre-treated standard grade, regenerated cellulose dialysis membrane MWCO 3500 Da was obtained from Spectrum® Labs. Mouse fibroblast cells (NIH 3T3) were kindly provided by Dr. Jürgen Schnekenburger (Biomedical Technology Center of the Medical Faculty Münster, Germany). Dulbecco's Modified Eagle's Medium (DMEM) high glucose, L-Glutamine and Penicillin Streptomycin were purchased from Gibco® Life Technologies. Ethanol and Hexamethyldisilazane ≥ 99% (HMDS) were bought from Sigma Aldrich.

2.2. Experimental methods

2.2.1. Synthesis of Ficoll methacrylate

Ficoll® PM 70 (1.00 g) was dissolved in a minimum amount of LiCl/DMF (10 wt%) in a quickfit test tube and heated at 90 °C under vacuum. The reaction solution was then cooled to 60 °C, after which 75 μL of TEA was added under vigorous stirring for 15 min. Methacrylic anhydride (1.59 μL) was added and the reaction mixture was stirred at 60 °C for 18 h. The reaction mixture was precipitated several times into cold acetone and the product obtained after decantation and drying under vacuum. The powder obtained was dialyzed against distilled water for 48 h using Pre-treated Standard Grade, regenerated cellulose dialysis membrane MWCO 3500 Da (Spectrum® Labs). After dialysis, the solution was freeze-dried to obtain the methacrylated Ficoll® PM 70 polymer.

2.2.2. Preparation of hydrogels via crosslinking of Ficoll methacrylate

5 mL of Ficoll® PM 70-MA solution (5 wt%) was prepared in distilled water followed by addition of 3.17 μL of EGDMA, 100 μL of TEMED (10 wt%) and APS (10 wt%). The solution was gently swirled to mix all the reagents and allowed to stand at room temperature until the hydrogel is formed. The obtained hydrogel was immersed in distilled water for one week while regularly changing the water to remove any unreacted chemicals. It was air dried until constant mass. The fraction of EGDMA was varied from 1 to 5% of the number of moles of methacrylate groups to obtain hydrogels of different degree of crosslinking.

2.2.3. Preparation of hydrogels in the presence of porogen

The porogens (sucrose or mPEG5000) were mixed initially with all reagents as in the previous paragraph and crosslinking allowed to proceed. Hydrogels were washed several times with water for a week to allow porogen molecules to leach out of the matrix and this was confirmed by comparing the mass and IR spectra of hydrogels prepared with and without porogens. The concentration of porogens was varied to obtain a series of hydrogels.

2.2.4. Swelling studies and swelling kinetics

Hydrogel samples of equal mass were placed in 10 mL of distilled water at RT. At regular time intervals, the hydrogel was removed from the medium and slightly blotted with an absorbent paper to remove the excess water on the surface and its mass was measured. The experiment was carried out till equilibrium swelling. The swelling percentage (% SW) was calculated using Eq. (1) [31,32]. The swelling ratio (Q) was calculated according to Eq. (2).

$$\%SW = \frac{(Mt - Mo)}{Mo} \times 100 \quad (1)$$

$$Q = \frac{Ms}{Mo} \quad (2)$$

where M_t is the mass of hydrogel at time t, M_o the initial mass of hydrogel and M_s the mass of hydrogel at equilibrium.

2.2.5. Dye loading by soaking

50 ± 5 mg of hydrogel was immersed in 5 mL of dye solution prepared in distilled water and kept in the dark for 1 week and the dye uptake was monitored by UV spectroscopy on a daily basis. Four dyes were used as model compounds to study the uptake capacity of the hydrogels, namely: NBA and BG, which are cationic in nature, and FSS and SRB, which are anionic dyes. Absorbance values of bands at 635 nm, 625 nm, 490 nm and 564 nm were recorded for NBA, BG, FSS and SRB, respectively. The percentage of dye loading into the hydrogel was calculated according to Eq. (3). When the loading experiment was over (after 7 days), the hydrogels were allowed to dry at RT.

$$\%Dye\ Loading = [(Co - Ct)/Co] \times 100] \quad (3)$$

where C_o is the concentration of the dye initially and C_t is the concentration of the dye at time t.

2.2.6. Dye entrapment during crosslinking

250 mg of Ficoll methacrylate was dissolved in 5 mL of dye solution prepared in distilled water. The same dyes as mentioned above were used (60–85 μM). The crosslinker EGDMA was added followed by addition of the initiators APS/TEMED. The hydrogel was allowed to form and immersed in distilled water for 1 week by changing the water daily. It was then allowed to dry at RT until constant mass.