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Biocompatible cryogels of thermosensitive polyglycidol derivatives with ultra-rapid swelling properties

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ABSTRACT

Novel thermosensitive macroporous cryogels, based on various hydrophobically modified high molar mass (HMM) polyglycidol precursors, were synthesized using the UV-irradiation technique. The method involved the preparation of a semi-dilute aqueous solution of thermosensitive poly(glycidol-co-ethyl glycidyl carbamate) (PGL-Et), subsequent freezing at a moderately negative temperature (−20 °C) and irradiation with UV light. All PGL-Et cryogels had a spongy-like structure of smooth polymer walls surrounding interconnected macroscopic pores. Consequently, the cryogels exhibited temperature triggered, reversible, ultra-rapid volume phase transition (VPT) from a swollen to deswollen state within 20–25 s. The VPT temperature of the PGL-Et cryogels was strongly dependent on the degree of modification of the PGL precursors and it decreased proportionally with increased ethyl glycidyl carbamate content. The PGL-Et cryogels were used as a scaffold for skin cell (fibroblast) adhesion. Adhesion and proliferation tests indicated that the gels were good supports for cell cultivation.

1. Introduction

Considerable interest has been focused on super-macroporous cryogels due to their unique heterogeneous open porous structure, which significantly increases the equilibrium sorption properties and allows unhindered diffusion of solutes, nano-particles and even micro-particles [1]. These materials have been attractive for many applications, especially in biomedicine and biotechnology for drug immobilization, biomolecules and cells or for bioseparation [1–3].

Cryogels are formed as a result of consequent freezing, cross-linking and thawing of monomers or linear polymers dissolved most often in water. During the cryogenic treatment process, most of the water forms ice crystals, whereas bound water and soluble substances accumulate in a non-frozen liquid microphase (NFLMP). The gel is formed in this liquid microphase and the ice crystals act as porogens.

The synthesis of polymeric hydrogels, based on the so-called "smart" polymers, have recently been addressed in numerous studies. These hydrogels undergo significant changes of their swelling properties with small changes in environmental conditions near critical temperature, pH, etc. [4,5]. In particular, thermosensitive hydrogels were among the most widely investigated representatives of smart hydrogels. Hydrogels of poly(*N*-isopropylacrylamide) (PNIPAAm), the most popular thermosensitive polymer, undergo reversible volume phase transition (VPT) from a swollen to deswollen state at a temperature of approximately 33 °C [6].

Special interest has also been paid to the "smart" macroporous cryogels due to their numerous advantages. The hydration–dehydration behavior of cryogels is much more rapid compared to conventional hydrogels obtained from

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the same polymer. This behavior has been attributed to the regular, spongy-like structure of the cryogel that contained a large amount of free water. During hydration/dehydration of the cryogel, the oriented and interconnected pores, with their very smooth wall interfaces, dramatically facilitated the diffusion of water and heat exchange. Comparing the macroporous cryogel of PNIPAAm with its hydrogels demonstrated these processes [7–9].

In the last decade, numerous cryogels based on high molar mass (HMM) polymers have been prepared by employing the UV-irradiation technique [10–12]. The advantages of UV irradiation include very low capital outlay and extremely short times for efficient gel formation. Thus, cryogels of poly(ethylene oxide) [10] and various cellulose derivatives [11,12] were prepared using only 2 min of UV irradiation (irradiation dose rate of 5.7 J/cm^2 min) of moderately frozen polymer systems in the presence of aromatic photoinitiator (4-benzoylbenzyl)trimethylammonium chloride (BBTMAC). Very recently, Petrov et al. reported on the synthesis of thermosensitive PNIPAAm and hydroxypropylcellulose cryogels via UV irradiation that exhibited reversible, ultra-rapid volume phase transition within 10–15 s [13].

Novel thermosensitive polymers have recently been obtained from high molar mass polyglycidol (PGL). The hydroxyl groups of a glycidol unit were hydrophobically modified through a reaction with ethyl isocyanate, leading to poly(glycidol-co-ethyl glycidyl carbamate)s [14]. These polymers showed phase separation temperatures, which may be adjusted in a very broad temperature range (10–90 °C). This temperature was varied by the copolymer composition and the presence of additives (salt, surfactants). It was also shown that the hydrogels based on the thermosensitive HMM polyglycidol derivatives could be obtained via chemical crosslinking [15]. Their swelling and shrinking behaviors were controlled by changing the composition of the copolymer precursor and by the network density.

In this paper, we report on the synthesis and characterization of novel thermosensitive cryogels based on HMM poly(glycidol-co-ethyl glycidyl carbamate) via UV irradiation. The influence of different synthesis conditions, including the copolymer concentrations and compositions as well as the irradiation dose on the preparation of cryogels and their swelling behavior is described. The swelling/shrinking kinetics of cryogels is investigated and compared to conventional hydrogels of poly(glycidol-co-ethyl glycidyl carbamate) obtained by chemical crosslinking of hydroxyl groups [15]. The biocompatibility of HMM poly(glycidol-co-ethyl glycidyl carbamate) (cryogel precursor) with fibroblasts is studied. Adhesion of fibroblasts to the cryogels is compared with both modified and pure HMM polyglycidol scaffolds.

2. Experimental

2.1. Materials

2,3-Epoxypropanol-1 (glycidol) and ethyl vinyl ether were purchased from Aldrich and distilled under reduced pressure and dry nitrogen atmosphere, respectively. Diethyl ether (POCh Gliwice) was dried over a Na/K alloy and distilled under dry nitrogen atmosphere prior to polymerization. Ethyl isocyanate, 98 % (Aldrich) was distilled under dry nitrogen atmosphere. *N,N′*-dimethylformamide (DMF) (POCh Gliwice) was initially dried over molecular sieves type 4 Å. It was then dried over CaH_2 and distilled under reduced pressure. A 1 M solution of diethyl zinc in hexane ($ZnEt_2$) (Aldrich), dibutyltin dilaurate 95% (DBTL) (Aldrich) and (4-benzoylbenzyl)trimethylammonium chloride (BBTMAC) (Aldrich) were used as received.

2.2. Synthesis and characterization of polymer precursors

The monomer, ethoxyethyl glycidyl ether (EEGE), was synthesized by reacting 2,3-epoxypropanol-1 and ethyl vinyl ether according to Fitton et al. [16] and distilled under reduced pressure to obtain a fraction with purity exceeding 99.8% (GC analyses). Then, EEGE was polymerized in bulk, with $ZnEt_2/H_2O$ (1:0.78) as the catalyst, using a procedure described elsewhere [14,17]. Polyglycidol was obtained by hydrolysis of poly(ethoxyethyl glycidyl ether) using 3 M HCl. Hydrophobically modified PGL random copolymers were prepared by reactions of different amounts of ethyl isocyanate (EtI) with the hydroxyl groups from glycidol units in the presence of dibutyltin dilaurate as a catalyst, in DMF as described previously [14]. The molar mass and molar mass dispersity of the polyglycidol were determined by SEC using a multiangle light scattering detector ((λ = 658 nm) DAWN HELEOS, Wyatt Technology) and a refractive index detector (Δ-1000 RI (λ = 620 nm), WGE DR Bures) [14]. SEC measurements were performed at 45 °C in DMF with 5 mM LiBr (POCh, Gliwice, Poland) at a nominal flow rate of 1 ml/min. The column set used for SEC measurement consisted of a PL gel guard column, two PL gel MIXED-C columns from Polymer Laboratories and a 100 Å PSS GRAM column from PSS. The ^{1}H NMR spectra of the polyglycidol and its hydrophobically modified derivatives were recorded in D_2O using a Bruker Avance Ultra-Shield spectrometer operating at 600 MHz. The cloud points of the hydrophobically modified polyglycidol precursors were measured using a Jasco V-530 UV–VIS spectrophotometer according to procedures described in Ref. [14].

2.3. Synthesis of cryogels

An appropriate amount of each copolymer was dissolved in distilled water under stirring to obtain a homogeneous aqueous solution (1–5 mass%). Given amounts of photoinitiator (BBTMAC, 5 mass% with respect to polymer) was added under stirring at room temperature. The resulting homogeneous solution was poured into Teflon dishes (20 mm diameter), forming a 4 mm thick layer, which was then kept in a freezer at −20 °C for 2 h. The frozen samples were irradiated with a full spectrum UV–vis light using a Dymax 5000-EC UV curing equipment with a 400 W metal halide flood lamp for 2–6 min (irradiation dose rate: 5.7 J/cm^2 min; input power: 93 mW/cm^2).

2.4. Measurements of gel fraction yield and degree of swelling

The gel fraction (GF) yield and degree of swelling (DS) of the cryogels were determined gravimetrically.