European Polymer Journal 44 (2008) 2962–2970

Contents lists available at ScienceDirect

European Polymer Journal

journal homepage: www.elsevier.com/locate/europolj

Studies on copolymerization of *N*-isopropylacrylamide with poly(ethylene glycol) methacrylate

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ARTICLE INFO

Article history:
Received 25 August 2007
Received in revised form 3 June 2008
Accepted 18 June 2008
Available online 25 June 2008

Keywords:
N-isopropylacrylamide (NIPAAm)
Photoinitiators
Cell culture
Hepatic cancer cell lines

ABSTRACT

The paper describes the preparation and characterization of cross-linked homopolymers and copolymers of *N*-isopropyl acrylamide (NIPAAm) with poly(ethylene glycol) methacrylate (PEGMA, M_n = 526 g/mol). Several copolymer samples were prepared by taking varying amounts of monomers i.e. NIPAAm and PEGMA in the initial feed using hydrophilic (IRGACURE-2959) and hydrophobic (DURACURE-1173) photoinitiator. In order to investigate the effect of reaction conditions, copolymers were prepared below or above the lower critical solution temperature (LCST) using water or water:ethanol (50:50) as solvent and by varying the amounts of cross-linker. Hydrogels prepared under varying reaction conditions were characterized for its swelling behaviour (using optical microscope), phase transition temperature (using DSC) and morphology (using SEM). As expected LCST increased from 35 to 39 °C as PEGMA content in copolymers increased from 1 to 20% (w/w). However, the morphology of hydrogels was found to be independent on the reaction conditions.
Copolymer films having an optimum combination of swelling and performance properties were evaluated as switchable cell culture membranes. Hepatic cancer cell lines (Hep G-2) was used to study the cell growth and detachment. Cell growth and detachment were found to be dependent on the copolymer composition. Cell viability was found comparable to trypsin which also supports application of these films as cell culture membrane.
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1. Introduction

Smart or intelligent polymer networks show sharp changes in response to physical stimuli like pH, temperature, ionic strength, electric or magnetic field. Among many intelligent polymers poly(*N*-isopropyl acrylamide) (PNIPAAm) is one of the extensively studied smart polymer and demonstrate a lower critical solution temperature (LCST) of about 32 °C in aqueous medium. It undergoes a sharp coil to globule transition in water above the lower critical solution temperature (LCST = 32 °C) i.e. changing from a hydrophilic state to a hydrophobic state above LCST. The main mechanism of phase separation is thermally induced release of water molecule bound to isopropyl side groups, resulting in increased inter- and intramolecular hydrophobic interactions between isopropyl groups above LCST. This unique property is widely used in drug delivery, tissue engineering and biotechnology [1–7]. A detailed review of the transition behaviour can be found in the literature [8].

Recently there has been a growing interest of using smart polymers in cell culture systems. Cultures of vital cells and tissues are widely used and have become powerful tools in exploring the production of bioactive compounds and screening novel pharmaceuticals. The use of

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doi:10.1016/j.eurpolymj.2008.06.020

cell models can replace the use of experimental animals. Various type of cells can adhere, spread and proliferate at 37 °C and at temperature below LCST, the cultured cells can detach from hydrophilic surface without the incorporation of chemicals/enzymes that could damage the cells. However conventional systems involve application of proteolytic enzymes (e.g. trypsin). These proteolytic enzymes cause cell damage by hydrolyzing functional proteins [9]. Several researchers have thus used hydrophilic/hydrophobic property alteration of polymer substrates to create switchable cell culture systems [10,11]. Ulbricht et al. has modified PAN-UF membrane (polyacrylonitrile ultra filtration membrane) by graft copolymerization using sequential and simultaneous photopolymerization method [12]. UF membranes with sufficient degree of modification showed little protein adsorption and no fouling due to BSA solution. Kwon et al. observed rapid cell sheet detachment from tissue culture polystyrene surface (TCPS) modified by grafting of PNIPAAm using electron beam irradiation [13]. Several researchers have observed that cell sheet detachment can be accelerated by incorporation of a hydrophilic moiety in to the bulk hydrogel, which will accelerate swelling deswelling characteristics probably due to the formation of water channels. Therefore it was considered of interest to investigate systematically the copolymerization of NIPAAm with varying amounts of PEGMA and evaluate their behaviour in cell culture studies.

This paper describes the copolymerization of NIPAAm with varying amounts of PEGMA using photopolymerization techniques and presents preliminary studies on cell adhesion and detachment. Several copolymer samples were prepared by taking varying amounts of PEGMA comonomer i.e. in the range of 1–20% (w/w), and *N*,*N*′ methylene bisacrylamide (BIS, cross-linker) in the initial feed. In order to investigate the effect of reaction parameters, copolymers were prepared using different solvent systems i.e. water, water:ethanol (50:50), varying temperatures i.e. above and below phase transition temperature and varying cross-linker concentration ranging from 1 to 4% (w/w). The effect of reaction conditions and the copolymer composition on swelling and thermal behaviour was evaluated. Morphological characterization was done using scanning electron microscopy (SEM). Cell sheet attachment and detachment studies were performed on selected samples.

2. Experimental

2.1. Materials

N-isopropyl acrylamide (NIPAAm, Aldrich) was purified by recrystallisation using hexane. *N*,*N*′-methylene bisacrylamide (BIS, Aldrich), 1-[4-(2-hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-propane-1-one (IRGACURE-2959, hydrophilic photoinitiator, Aldrich); 2-hydroxy-2-methyl propiophenone (DURACURE-1173, hydrophobic photoinitiator, Aldrich); poly(ethylene glycol) methacrylate (M_n = 526 g/mole) (macromonomer, Aldrich); Hep G-2 cancer cell lines; fetal calf serum (Sigma chemicals, St. Louis, USA); trypsin (Sigma chemicals); streptomycin; neomycin; penicillin and ethanol were used as received. Deionised water was used in all the experiments.

2.2. Polymerization

Copolymerization of NIPAAm with PEGMA was carried out in solution using IRGACURE 2959 (hydrophilic) or DURACURE-1173 (hydrophobic) photoinitiator (2% w/w), BIS as cross-linker and water or water:ethanol (50:50) as solvent as reported earlier [14]. Several copolymer samples were prepared by taking varying amounts of PEGMA in the initial feed ranging from 1 to 20% w/w. Polymer solution was purged with argon for 5 min and poured in to the glass reactor having 500 μm spacer and covered with glass plate. Photopolymerization was carried out for 3 min. Polymeric films were washed with distilled water extensively for one week to remove unreacted monomer.

Polymerization was carried out below LCST (7 °C) and above LCST (40 °C) using hydrophilic photoinitiator and water as solvent. Polymer samples were also prepared at 7 °C using hydrophobic photoinitiator and water:ethanol mixture (50:50) as solvent. The system was homogeneous when water was used as solvent at 7 °C whereas it was heterogeneous when water (at 40 °C) or water:ethanol (50:50) (at 7 °C) was used as solvent.

Copolymer gels have been designated as NPG followed by numerical suffix indicating the wt% of PEGMA in the initial feed. For example, copolymers prepared by taking 1, 5 and 10% (w/w) of PEGMA have been designated as NPG-1, NPG-5 and NPG-10, respectively. The copolymer samples prepared under heterogeneous conditions i.e. using water (at 40 °C) or water: ethanol (50:50) (at 7 °C) are designated by adding letter "H" and "WE" within parenthesis i.e. NPG-5 have been designated as NPG-5(H) and NPG-5(WE), respectively.

In order to evaluate the effect of cross-link density, NPG-10 copolymer was prepared by taking varying amounts of BIS ranging from 1 to 4% (w/w). The samples have been designated as NPG-10 followed by suffix within parenthesis representing the amount of BIS. For example NPG-10 prepared using 2 and 4% (w/w) of BIS has been designated as NPG-10(2) and NPG-10(4) respectively. Homopolymer gels of NIPAAm are designated as PNIPAAm, PNIPAAm(H) and PNIPAAm(WE), respectively. Tables 1 and 2 show the detailed sample designation of various copolymers prepared by photopolymerization.

2.3. Characterization

For swelling studies, circular samples having identical diameter of 4.5 mm, were cut from the films (kept at 50 °C for 15 min) using a standard method. The circular film samples were then immersed in a thermostated chamber. Temperature of the bath was increased slowly and the gel diameter was measured at different temperature using optical microscope (Hund). During the measurement care was taken to hold the samples at a particular temperature for at least 10 min so that it attains the equilibrium swelling temperature. The change in volume $(d/d_0)^3$ was calculated as a function of temperature (d_0 is

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