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Cytotoxicity of thermosensitive polymers poly(*N*-isopropylacrylamide), poly(*N*-vinylcaprolactam) and amphiphilically modified poly(*N*-vinylcaprolactam)

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Abstract

Thermosensitive polymers poly(*N*-isopropylacrylamide) (PNIPAM), poly(*N*-vinylcaprolactam) (PVCL) and PVCL grafted with amphiphilic poly(ethylene oxide) (PEO) chains (PVCL-graft-$C_{11}EO_{42}$) were prepared and characterized and their putative cytotoxicity was evaluated. The cytotoxicity of these thermosensitive polymers and their monomers was investigated as a function of polymer concentration, incubation time and incubation temperature by using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) cytotoxicity tests in Caco-2 and Calu-3 cell cultures. Also, the influence of the chain end functionality on toxicity was examined. Viability (MTT) and cellular damage (LDH) of the cells were shown to be dependent on the surface properties of the polymers, hydrophilicity or hydrophobicity. Hydrophilic PVCL and PVCL-graft-$C_{11}EO_{42}$ were well tolerated at all polymer concentrations (0.1–10.0 mg/ml) after 3 h of incubation at room temperature and at physiological temperature (37 °C). The more hydrophobic PNIPAM induced more clear cellular cytotoxicity at 37 °C. The monomers *N*-isopropylacrylamide and vinylcaprolactam and PEO-macromonomer showed dramatically higher cytotoxicity values with respect to the corresponding polymers. Cell damage was directly dependent on concentration, temperature and incubation time.

Keywords: Cytotoxicity; Thermosensitive polymers; PVCL; PNIPAM; MTT test; LDH test

1. Introduction

Thermally responsive polymers and their use in biomedical applications are widely investigated nowadays. Thermally responsive polymers exhibit a lower critical solution temperature (LCST), below which the polymers are soluble. When the temperature is raised above the LCST, the polymers undergo a phase transition, collapse and, further, form aggregates. This phenomenon is reversible and thus, when the temperature is lowered, the polymers become soluble again.

Poly(*N*-isopropylacrylamide) (PNIPAM) is the most extensively studied thermoresponsive polymer for therapeutic purposes. PNIPAM has an LCST value at approximately 32 °C [1]. It is soluble at room temperature and phase separates at body temperature (37 °C), so it is a potential candidate as a biomedical carrier either as linear polymer, hydrogel or copolymer [2–6]. PNIPAM has been used, for example, in drug targeting for solid tumors with local hyperthermia [7,8], in

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thermosensitive coatings or micelles for controlled release of the drug [9,10], and as a cell attachment/detachment surface [11,12]. In tablet coating, a controlled release of the drug has been achieved by changing the LCST value of the polymer either by salt concentration or by the amount of surfactant [13–15]. PNIPAM polymers have been used in eye drop preparations, where no in vitro cytotoxicity was found [16,17], and as a new embolic material in neurosurgery [18], where no acute toxicity in mice was noted.

Another temperature-responsive and biocompatible polymer that has been studied for therapeutic purposes is poly(*N*-vinylcaprolactam) (PVCL). The polymer is widely used in hair-care and cosmetic applications [19]. Applications of PVCL in the area of biomedical materials, in stabilization of proteases and in controlled drug delivery and drug release have been published for example by Peng and Wu [20], Markvicheva et al. [21] and Vihola et al. [22]. Also PVCL collapses when the temperature exceeds 32 °C [23] and, therefore, the thermosensitive PVCL has, presumably, similar characteristics as PNIPAM. PVCL is a chemical analogue of polyvinylpyrrolidone (PVP), a well-known and widely used pharmaceutical excipient [24–27]. However, some evidences of PVP dose- and time-dependent toxicity have been found recently [28]. To our knowledge, the evaluation of putative cytotoxicity of PVCL polymers has not been published previously. Biocompatibility of synthetic polymers has been improved by grafting hydrophobic polymers with hydrophilic chains, e.g., poly(ethylene oxide) (PEO) or with some other water-soluble polymers. The grafting creates steric repulsion against protein adsorption and, thus, increases the biocompatibility of the polymer [29].

The in vitro cytotoxicity of PVCL of various molecular weights, PVCL grafted with amphiphilic chains containing PEO segments (PVCL-graft-$C_{11}EO_{42}$), PNIPAM polymer and the corresponding monomers was investigated. The putative cytotoxicities of the monomers were compared; they were also compared with those of the corresponding polymers. The effect of phase separation on the cytotoxicity was determined; the tests were performed at 23 and 37 °C, below and above the LCST values of these polymers. The cytotoxicity assays were performed with two different cell cultures, intestinal Caco-2 and pulmonary Calu-3, which act as model epithelia for oral and pulmonary drug delivery, respectively. The cytotoxicity of the polymers was evaluated by two methods, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) tests, that indicate the cell survival and damage of cell membrane, respectively. The tests were performed as a function of incubation time and polymer concentration.

2. Methods

2.1. Materials

N-vinylcaprolactam (VCL, 98%, Aldrich Chemicals, Germany) and *N*-isopropylacrylamide (NIPAM, 99%, Acros Organics, Belgium) were purified by recrystallization from benzene. The amphiphilic PEO macromonomer, $MAC_{11}EO_{42}$ ($M_w = 2110$ g/mol), was prepared as described previously [30]. The macromonomer is composed of a hydrophilic PEO chain (42 ethylene oxide units), and a relatively short hydrophobic alkyl chain (11 methylene units). Reactive methacrylate is located at the hydrophobic end of the molecule (Fig. 1). The substance is readily water soluble although it has an amphiphilic nature. 2,2′-Azo-bis-isobutyronitrile (AIBN, Aldrich Chemicals, Germany) was purified by recrystallization from methanol. 2,2′-Azo-bis[2-methyl-*N*-(2-hydroxyethyl)propionamide] (VA-086, Wako Chemicals, Japan) was used as received. Deionized water was obtained from an Elgastat UHQ-PS water purification system (Elga Ltd., England). Tetrahydrofuran (THF) and hexane (both HPLC grade from Rathburn, Scotland), benzene (99.7%, Reidel-de-Haen, Germany) and dioxane (Lab Scan, Ireland) were used without further purification.

2.2. Polymerizations

Polymer samples were prepared by the following procedure. A solution of monomers in the selected solvent (Table 1) was degassed with nitrogen for 30 min. It was heated to 70 °C, unless otherwise indicated. As soon as the polymerization temperature was reached, a solution of the initiator was injected into the solution. At the end of the polymerization, the mixture was cooled down to room temperature and the polymer was isolated by precipitation. PVCL polymers (PVCL-330, PVCL-1300 and PVCL-1500 indicate the molecular

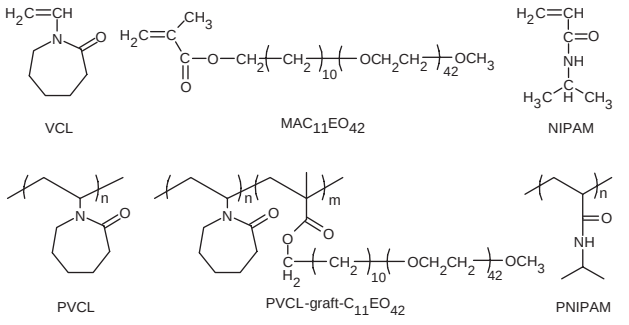

Fig. 1. Top: chemical structures of the monomers *N*-vinylcaprolactam (VCL), PEO-macromonomer ($MAC_{11}EO_{42}$) and *N*-isopropylacrylamide (NIPAM). Bottom: chemical structures of the polymers poly(*N*-vinylcaprolactam) (PVCL), poly(*N*-isopropylacrylamide) (PNIPAM), and the copolymer of *N*-vinylcaprolactam and PEO-macromonomer (PVCL-graft-$C_{11}EO_{42}$).