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pH-degradable PVA-based nanogels via photo-crosslinking of thermo-preinduced nanoaggregates for controlled drug delivery

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ABSTRACT

pH-Degradable PVA nanogels, which are prepared by photo-crosslinking thermo-preinduced PVA nanoaggregates in water without any surfactants or toxic organic solvents, are used for intracellular PTX release and anticancer treatment. These nanogels fast degraded at mildly acidic conditions with a pH-triggered PTX release, and the degradation products are only native PVA and poly(hydroxyethyl acrylate) (PHEA) as well as acetaldehyde without any toxic byproducts. The nanogel sizes could be tailored by different temperatures during the crosslinking process. The results of confocal microscopy and flow cytometry revealed that smaller nanogels exhibited enhanced internalization with MCF-7 cells than the ones treated with larger nanogels, by which the smaller PTX-loaded nanogels induced a more significant cytotoxicity against MCF-7 cells.

Graphic abstract: pH-Degradable PVA nanogels can be prepared by photo-crosslinking of thermo-preinduced nanoaggregates with tailored nanogel sizes given their pH-triggered PTX release and fast acid-degradation into native PVA and cell-compatible poly(hydroxyethyl acrylate) (PHEA) as well as acetaldehyde.

1. Introduction

Nanomedicines have conferred many advantages for cancer diagnosis and therapy [1–4]. The preclinical and clinical studies have demonstrated that advanced drug delivery nanosystems provide prolonged circulation time, efficient tumor-targeted accumulation via the enhanced permeability and retention (EPR) effect, reduced side effects, and improved drug tolerance, that has resulted in better drug bioavailability and therapy [5–9]. To meet the pharmaceutical requirements, biocompatible nanocarriers including liposomes, polymeric nanoparticles, micelles, and nanogels have been developed for delivery of various types of drugs for cancer treatment. Compared with other nanocarriers, nanogels with internally crosslinked three-dimensional (3D) structures show high water content, desirable chemical and mechanical properties, and a large surface area for multivalent bioconjugation [10–14]. They are able to stabilize bioactive compounds such as drugs, peptides/proteins, and DNA/RNA in their polymeric networks, and moreover, nanogels actively participate in the drug delivery process due to their intrinsic properties such as stimuli-responsive behavior, swelling and softness, to achieve a controlled drug release at the target site [15–18].

Poly(vinyl alcohol) (PVA) is a synthetic polymer with OH-hydrophilicity prepared by radical polymerization of vinyl acetate and followed with partial hydrolysis. Due to its unique properties including water solubility, multiple OH-groups for further decoration, and FDA-approved biocompatibility and low toxicity, the use of PVA as a biomaterial has attracted great attention in biomedical applications such as protein/enzyme immobilization, cell encapsulation in the form of micro/hydrogel scaffolds [19–22]. However, PVA nanostructures failed to meet the demand, especially in the field of nanomedicine area, which is mostly due to their inhomogeneous interior, high porosity, low drug affinity, and uncontrollable release behavior. The modification of OH-groups on PVA is largely considered to introduce more convenient sites for conjugation and chain extension using ester, carbamate, ether and acetal linkages [23]. For example: Kupal et al. reported that core–shell PVA-based microgels shielded with hyaluronic acid (HA) were prepared by "click" chemistry and inverse emulsion techniques for targeted local delivery of doxorubicin to adenocarcinoma colon cells (HT-29) [24]. We recently developed charge-conversional reducible PVA nanogels by combining nanoprecipitation and "click" chemistry for an enhanced cellular uptake towards universal tumor cells and efficient tumorous cytotoxicity against human cancer MCF-7 cells [25].

Much effort has been made towards the development of pH-sensitive nanocarriers for intracellular drug delivery, since there are natural pH gradients in the tumor tissue microenvironment (pH 6.5–7.2) as well as the endosomal/lysosomal compartments of tumor cells (pH 4.0–6.5) [26–32]. It is noteworthy that the strategy of pH-triggered drug release has been exploited to meet the challenges of various extra- and intra-cellular barriers towards successful cancer chemotherapy for drug release nanosystems. The acid-labile acetal linkage has been

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widely introduced to fabricate pH-sensitive nanostructures and networks for intracellular drug delivery, in which the acetal groups are relatively stable under physiological conditions, while rapidly hydrolyzed at a mildly acidic pH to release the encapsulated cargo [33–35]. Acetalization reactions were usually used to equip PVA chains with acrylamide groups and conjugated heterocyclic chromophores, as well as photoactive groups [36,37]. In this study, we developed pH-degradable nanogels based on acetal-linked PVA with defined shape and size for encapsulation of PTX and intracellular release (Scheme 1). These functionalized PVA materials could firstly form nanoaggregates in water by thermo-trigger, which was followed by photo-irradiation to produce nanogels with high stability under physiological conditions. These nanogels could degrade fast at a mildly acidic pH, and more interestingly, the degradation products are only native PVA and cell-compatible poly(hydroxyethyl acrylate) (PHEA) as well as acetaldehyde without any toxic byproducts. We investigated the thermo-transition behavior of the functionalized PVA, the stability and degradation of the nanogels, together with the in vitro drug release, size-dependent cellular uptake and tumorous cytotoxicity of PTX-loaded nanogels.

2. Experimental section

2.1. Materials and methods

Ethylene glycol vinyl ether (Aldrich, 97%), acryloyl chloride (Aldrich, 97%), triethylamine (Et_3N, Acros, 99%), 2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone (I2959, Aldrich, 98%), paclitaxel (PTX, Sigma, 97%), *p*-toluenesulfonic acid monohydrate (PTSA, Sigma-Aldrich, 98%) were used as received. Polyvinyl alcohol (PVA, Mowiol 3-85, M_w = 15,000 g/mol) was provided by Kuraray Europe GmbH (Germany). For cell culture experiments, MCF-7 cells (DSMZ, 115) were cultured in RPMI supplemented with 10% fetal calf serum, MEM nonessential amino acids, 1 mM sodium pyruvate, and 10 μg/mL human insulin. A549 cells (DSMZ, ACC 107) were cultured in DMEM supplemented with 10% fetal calf serum, and 2 mM glutamine.

^{1}H NMR spectra were recorded on a Bruker ECX 400. The chemical shifts were calibrated against residual solvent peaks as the internal standard. IR measurements were carried out on a Nicolet AVATAR 320 FT-IR 5 SXC that was equipped with a DTGS detector from 4000–600 cm^{-1}. The size of nanogels was determined by dynamic light scattering (DLS) at 25 °C using a Zetasizer Nano-ZS from Malvern Instruments equipped with a 633 nm He–Ne laser. Transmission electron microscopy (TEM) measurement was performed on Philips EM12 and operated at 100 kV with a nanogel concentration of 1 mg/mL. The TEM samples were prepared by dropping 5 μL of nanogel suspensions on the microscopical 200 mesh grids, which were placed on liquid nitrogen. After 5 min, the samples were lyophilized for TEM measurement.

2.2. Synthesis of vinyl ether acrylate (VEA)

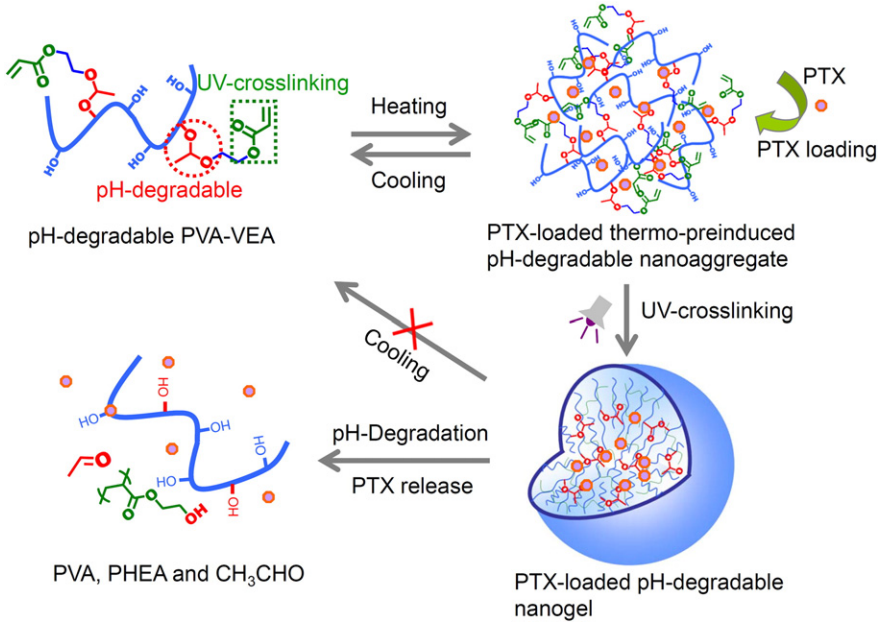
Ethylene glycol vinyl ether (20.00 g, 0.227 mol) and Et_3N (41.6 mL, 0.30 mol) were dissolved in dichloromethane (CH_2Cl_2, 300 mL), followed by a drop wise addition of acryloyl chloride (22.5 mL, 0.272 mol) at 0 °C. After 8 h, the reaction solution was extracted with $NaCO_3$ aqueous solution twice, and the organic phase was then dried over $MgSO_4$ and concentrated by rotary evaporation. The final product was collected by distillation under reduced pressure. Yield: 27.72 g (86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.60 (1H, CH_2═$\underline{CH}$–O–), 5.85–6.45 (3H, $\underline{CH_2}$═$\underline{CH}$–C(O)–), 4.40 (2H, –$\underline{CH_2}$–O–C(O)–), 4.05–4.20 (2H, $\underline{CH_2}$═CH–O–), 3.94 (2H, –$\underline{CH_2}$–CH_2–O–C(O)–); ^{13}C NMR (400 MHz, $CDCl_3$): δ 166.10 (CH_2═CH–$\underline{C}$(O)–), 151.50 (CH_2═$\underline{CH}$–O–), 131.40 ($\underline{CH_2}$═CH–C(O)–), 128.12 (CH_2═$\underline{CH}$–C(O)–), 87.15 ($\underline{CH_2}$═CH–O–), 65.77 (–$\underline{CH_2}$–CH_2–O–C(O)–), 62.79 (–CH_2–$\underline{CH_2}$–O–C(O)–).

2.3. Synthesis of VEA-functionalized PVA (PVA-VEA)

PVA (1.00 g, 17 mmol of OH group) was dissolved in anhydrous DMSO (100 mL), and then VEA (10, 20, and 40 mol% of OH group in PVA) and a catalytic amount of PTSA were sequentially added to the reaction to prepare PVA-VEA with different functionalities. After 6 h, the reaction was quenched by addition of Et_3N. The solvent was removed by dialysis against methanol, and then the polymer solution was concentrated by rotary evaporation. Finally, the polymer was isolated by precipitation in diethyl ether, and re-dissolved in water and freeze-dried.

2.4. Preparation of pH-degradable nanogels

PVA-VEA polymer dissolved in water with a concentration of 1.0 mg/mL containing 0.05 mg/mL of I2959 photo-initiator was kept



Scheme 1. Illustration of PTX-loaded PVA nanogels via photo-crosslinking of thermo-preinduced nanoaggregates, and pH-triggered degradation of nanogels to native PVA and poly(hydroxyethyl acrylate) (PHEA) as well as acetaldehyde.