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Enhance chemotherapy efficacy and minimize anticancer drug side effects by using reversibly pH- and redox-responsive cross-linked unimolecular micelles

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ABSTRACT

Chemotherapy side effects elimination while keeping the original therapeutic advantages has been of extensive interest over last decades. We herein develope a new pH and redox-sensitive nanogel for glutathione-mediated intracellular drug delivery employing disulfide crosselinked unimolecular micelles. We developed H40-polycaprolactone-b-polyacrylic acid-b′-methoxy poly(ethylene glycol)/poly(-ethylene glycol)-folate (i.e., H40-PCL-(b-PAA-b′-MPEG/PEG-FA)$_2$) unimolecular micelles with targeting moieties on the periphery. It was then crosslinked with cystamine in order to generate a pH and redox-sensitive nanogel. Dynamic light scattering (DLS) was performed to study pH-dependent nanogel sizes. We applied Paclitaxel, a hydrophobic anticancer drug, into delivery system and examined triggered release behaviors at different pHs upon DTT buffer exposure. Our results demonstrated that DTT presence as reductive agent within acidic environment is essential for drug release. This may potentially reduce unwanted drug release at non-cancerous acidic tissues leading to eliminated side effects. We examined biocompatibility and cytotoxicity of free nanogel and PTX-loaded nanogel to normal and cancer HeLa cells using MTT assay. Nanogel HeLa cell uptake was confirmed by Fluorescein loading into the gel followed by fluorescent microscope imaging. Our novel strategy would have profound implications in both enhanced chemotherapy efficacy and minimized side effects.

1. Introduction

Cancer is most complex and challenging diseases and is one of the leading causes of mortality worldwide. Chemotherapy is often prescribed as a hopeful treatment for cancer versus other limited treatments such as radiation therapy and surgical resection [1].

In chemotherapy, the cancerous cell is killed or stops dividing by application of toxic drugs. The toxic drugs rapidly prevent proliferation of cells and protect them from metastases. It must be noted that through treatment via toxic drugs not only cancer cells are targeted but also a group of healthy cells, such as cells in the hair follicles, oral mucosal cells, and bone marrow which have rapid proliferation will be targeted as well [2]. This inadequate targeting and nonspecific tissue bio-distribution of chemotherapeutic drugs can cause severe side effects such as hair loss [3], nausea [4], diarrhoea [5] and sexual dysfunctions [4] to patients. Thus, improving the efficacy of chemotherapy and decreasing the anticancer drug side effects are an important field of research in cancer therapy.

In recent years, the use of nanocarriers as chemotherapeutic drug delivery systems (20–200 nm) have attracted great attention as novel therapeutic modalities for cancer treatment [6]. Polymeric nanocarriers, are the most widely investigated nanotechnology platform for cancer therapy due to their ability to be chemically modified. These nanoparticles have good structural variability and can be tailored in to various categories such as nanospheres [7], liposomes [8], micelles [9–11], nanogels [12–19] and vesicles [20].

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Polymeric micelles based on amphiphilic block copolymer are one of the most common and important carriers owing massive potentials as a delivery vehicle for anticancer drugs. Nanomicelles can load hydrophobic drugs, have circumvent renal excretion and enhance circulation time in the body compared to the free drugs. These features can lead to increased aggregation of the drug loaded micelles in the cancerous tissue due to the enhanced permeability and retention (EPR) effect (passive targeting) [21,22]. The actively enhanced cellular internalization (active targeting), can also be obtained through small compound-receptor-mediated endocytosis by incorporation of small compounds such as folic acid (FA), peptides, hormones and antibodies to the surface of nanomicelles [21,22]. On the other hand, by using of stimuli-responsive nanomicelles, the rate of intracellular drug release after the penetration of nanoparticles to the cytoplasm of cancerous cells increases and leads to sufficient concentration of active drug within the cells. As a result this will reduce drug resistance of cells, decrease drug dosages, minimize unwanted side effects and also enhance the therapeutic efficacy [7,8]. The relatively low pH (~5–6.7) and high temperature (~40 °C) of cancer tissue as well as high level of reduced glutathione (GSH) in cancerous cells (10.0 mM) makes them distinguished from healthy tissues with a physiological condition of (T = 37 °C, pH ~7.4 and GSH ~2.0–20.0 μM) [9,10]. The difference in these variables can assist engineers to develop carriers which are sensitive to pH [11,12], temperature [13,14], and redox potential [15–21] or a combination of them [22,23] to trigger anticancer drug release at cancerous site. However, these physically assembled micelles have some drawbacks that limit their application as injectable nanocarriers. The low in vivo structural stability and disintegration of multi-molecular micelles after injection in biological milieu due to their dilution below its critical micelle concentration (CMC) is the main drawback of micelles. This will lead to premature drug release during blood circulation and unwanted side effects on normal cells [23]. Use of unimolecular micelles or introducing cross-links between the polymer chains in micelles, are two main strategies for stabilizing of micelles against dilution [25]. Unimolecular micelles based on hyperbranched dendrimers due to high-functionality and many nanocavities for drug encapsulation, have attracted great attention as a promising drug delivery system [23]. In our previous study, we developed a new pH-sensitive unimolecular micelles based on Boltron® H40 as core and poly(acrylic acid) as pH-sensitive arms [24]. The drug-loaded unimolecular micelles was relatively stable at physiologic conditions but susceptible to acidic environments and triggered the release of encapsulated drugs. However, it seems that this system requires more modification to enhance its selectivity to cancer cells. As the less discussed topic, some infections such as inflammation that are associated with more pathological events (such as injury, bacterial infection, etc.) can lower the pH of tissues similar to pH of cancer tissue [25]. Also, the vascular permeability (EPR effect) has been observed in many inflammatory conditions and used for passive targeting of anti-inflammatory drug delivery systems [26]. Therefore, if there is such infections in patients, the pH-sensitive nanocarriers can aggregate and release its payload drugs in inflamed tissue instead of cancer tissue and lead to sever side effects. The selectivity of drug delivery systems to cancer tissue can be increased by using of redox sensitive linkage in nanomicelles backbone [27]. Pang et al. synthesized bioreducible amphiphilic multiarm hyperbranched copolymer (H40-star-PLA-SS-PEG) based on Boltorn® H40 core, poly(L-lactide) (PLA) inner-shell, and poly(ethylene glycol) (PEG) outer-shell with disulfide-linkages between the hydrophobic and hydrophilic moieties. In vitro release studies revealed that the PEG outer-shell was detached from DOX-loaded micelles under reduction stimulus, which led to severe micelles aggregations and rapid drug release [28]. Liu et al. also synthesized reduction-sensitive shell-sheddable unimolecular micelles with hydrophilic polyphosphate arms (H40-star-PLA-SS-PEP). The cleavage of disulfide linkages in the presence of reducing agents such as glutathione (GSH) and detach of hydrophilic shell of micelles resulted to large aggregation of micelles and faster DOX release in cells [29]. However, to the best of our knowledge, unimolecular micelles based on Boltron® H40 containing unique disulfide bonds as redox-sensitive cross-linker for glutathione-mediated intracellular drug delivery has not yet been reported. In this study and in continuation of our previous study [24], to increase the selectivity of nanoparticle to cancer cells and also increase the rate of endosomal drug release, a new type of pH-sensitive unimolecular micelles cross-linked with redox-sensitive cross-linkers was developed. The cross-linkers in micelles can act as a barrier to drug release and thus, the introduction of stimuli-sensitive degradable linkage in cross-linker, is an essential and attractive method for controlled drug release from micelles [30–32]. The resultant nanogel can be stable in blood stream, inflamed conditions and extracellular milieu without significant structural deformation. Drug release will be accelerated through cleavage of redox-sensitive cross-linkers and shrinkage of resultant pH-sensitive micelles in the reducing and acidic environment of lysosome at the cancerous cells (Scheme 1). To investigate the drug loading content and drug release pattern, the hydrophobic anticancer drug, paclitaxel, was loaded as a model drug in nanogel. The effect of pH and reductive agent (DTT) on release profile were determined. Subsequently, the cell-uptake and cell-toxicity of drug-loaded nanogel were investigated.

2. Experimental

2.1. Materials

H40 (Perstorp Chemicals) purified with acetone and tetrahydrofuran (THF). Poly(ethylene glycol) and monomethoxy poly(ethylene glycol) (PEG and MPEG, Mn = 2000 g/mol) (Fluka) dried by azeotropic distillation by using anhydrous toluene. Tert-butyl acrylate (tBA) (Sigma) and ε-Caprolactone (CL) (Sigma) were purified with reflux over CaH_2 and vacuum distillation. Dithiothreitol (DTT) (Sigma) and commercial PTX formulation Tarvexol® (Sandoz Pharmaceutical Co., Argentina) was used as received. All other materials were purchased from Aldrich and used as received. THF, toluene, dichloromethane (DCM) and Triethylamine (TEA) were dried and distilled just prior to use. All other solvents were used as received. Alkynyl-methoxy poly(ethylene glycol), alkynyl-poly(ethylene glycol)-folate (FA-PEG) and 2,2-bis(methyl-α-bromoisobutyrate) propionic acid (BBPA) were synthesized in our lab and described previously [33]. For biocompatibility and cytotoxicity analysis, the amniotic epithelial (AE) cells and HeLa cancer cells were obtained from elective Cesarean and Pasteur Institute (Tehran, Iran), respectively. Fetal calf serum (FCS), RPMI 1640 and Dulbecco's Modified Eagle's Medium (DMEM)/F12 were obtained from GIBCO Invitrogen Corporation. Epidermal growth factor and (EGF)3-(4,5 Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma.

2.2. Instrumentation

The BRUKER DRX-300 AVANCE spectrometer was used to record ^{1}H NMR spectra of synthesised materials. The 102 MB BOMEM apparatus was used to record the FT-IR spectra. The Waters 2690D gel permeation chromatographic (GPC) system equipped with refractive index detector (Waters 2410) and column of AGILENT PLgel 10 μm 300 × 7.5 mm (500 A, 103 A, 104 A) was used to determine the molecular weights and molecular weight