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A nanogel of on-site tunable pH-response for efficient anticancer drug delivery

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ABSTRACT

A smart, soft and small nanoparticulate drug carrier that can efficiently transport therapeutics into tumor cells to control the intracellular drug concentration will enable major advancements in cancer therapy. To facilitate a remote modulation of the intracellular pH-regulated drug release, we have designed a new class of pH-responsive chitosan-based nanogels (<200 nm) by the physical interpenetration of chitosan chains into a nonlinear poly(ethylene glycol) (nonlinear PEG) chain network. The resultant PEG-chitosan nanogels not only respond to the changes in environmental pH over the physiologically important range of 5.0–7.4, but – more importantly – also enable us to remotely modulate the pH response by external cooling/heating. The nanogel, as well as the nanogel loaded with a model anticancer drug 5-fluorouracil (5-FU), is capable of varying its surface charge from nearly neutral to positive around tumor extracellular pH (~6.0–6.2) to facilitate cell internalization. Subsequently, the significantly increased acidity in subcellular compartments (~5.0) can trigger 5-FU release from the endocytosed drug carriers. While this nanogel serving as a drug carrier exhibits a reduced toxicity in combined chemo-thermo treatments, it has shown significantly enhanced therapeutic efficacy in combined chemo-cryo treatments of the model B16F10 melanoma cells, indicating its great potential for cancer therapy.

1. Introduction

Nanoparticle (NP)-based drug carriers have shown exciting efficacy for cancer treatments due to their improved pharmacokinetics and biodistribution profiles via the enhanced permeability and retention (EPR) effect [1]. However, the EPR effect can only enhance the accumulation of NPs in tumor tissues, whereas the poor cellular internalization and insufficient intracellular drug release always limits the dosages of anticancer drugs to the level below the therapeutic window, which hampers the efficacy of cancer chemotherapy [2]. To address this issue, stimuli-responsive drug carriers have been attempted to improve the drug bioavailability [3–5]. Of these carriers, pH-responsive nanogels is the most frequently used, as pH values in different tissues and cellular compartments vary tremendously. For example, many pathological processes in tumor tissue and intracellular endosome/lysosome are accompanied with local pH decrease by 1–2.5 pH units in comparison with that (pH 7.4) of blood and normal tissues [6–8]. Various pH-responsive nanogels have been developed for pH-triggered drug delivery [8–16]. A key attribute of the pH-responsive nanogels as drug carriers is their ability for pH-regulated drug delivery, minimizing side effects, and improving therapeutic efficacy of conventional drugs. Other advantages include simple synthesis, easy functionalization with targeting ligands and unique physical properties common to living tissues [17]. However, those nanogels display a fixed pH-responsive phase behavior, impairing the efficacy in the control of drug delivery. In most current cases, to obtain multiple pH-regulated drug release profiles, various nanogels of different pH-responsive phase behaviors should be synthesized by changing the parameters for the synthesis, including the selection of ionizable reagents from a number of candidates with different pK_a values and their feeding ratio, which requires additional workload because the swelling onset and swelling degree of the gels are directly related to their components and are poorly predictable from the existent theory [3]. The development of pH-responsive nanogels, which can not only respond to the changes in environmental pH but also allow on-site modulation of the pH response for enhanced drug delivery, has not been reported.

As a proof-of-concept, we described herein a chitosan-based nanogel of on-site tunable pH response as a nanoparticulate drug carrier. Chitosan is chosen since it has ionizable glucosamine in its molecular structure and has been extensively used in biomedical fields including drug/gene delivery and tissue engineering [18]. While it has been possible to obtain chitosan-based gels of different pH-responsive phase behaviors by tailoring their topology

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and molecular structure or introducing a second polyelectrolyte of desirable pK_a value [12,14,19,20], rational design of chitosan-based nanogels with on-site tunability of the pH-response by using a remote trigger possesses a great challenge. Theoretically, it is predicted that the incorporation of temperature-responsive phase transition could allow us to modulate the pH-responsive swelling degree of the gels by changing the surrounding temperature [3,21]. Although poly(*N*-isopropylacrylamide) (PNIPAM) or other temperature-responsive polymers have been incorporated into chitosan-based gels to enable pH and temperature dual response [22–25], unfortunately, only a number of examples drew the effect of surrounding temperature on the pH response. Heras's group synthesized a unique chitosan hydrogel from alkali chitin (degree of acetylation = 79 mol.%) [26]. They found that the hydrogel at pH = 7.6 can experience a two-fold increase in the swelling state from those initially attained at 25.0 °C upon changing the temperature to 2.0 °C; on heating back to 25.0 °C, the hydrogels deswelled reversibly to the initial state. Jeong's group reported a pH and temperature dual-responsive aqueous solution of poly(ethylene glycol)-poly(alanine) grafted chitosan [27]. When the pH of the solution increased from 3.0 to 6.5 and to 9.0, its sol–gel transition temperature would be changed from 17.0 °C to 27.0 °C and to 32.0 °C, and the modulus of the in situ-formed thermal hydrogel at 37.0 °C would be changed from 396 Pa to 241 Pa and to 43 Pa.

In this paper, we report the design of a new class of chitosan-based nanogels by the physical interpenetration of chitosan chains into a nonlinear poly(ethylene glycol) (termed nonlinear PEG) chain network, resulting in a semi-interpenetrating (semi-IPN) structured PEG-chitosan nanogel (<200 nm; Fig. 1). The non-linear PEG, a polymer containing short oligo(ethylene glycol) side chains, combines the advantages of linear PEG (i.e. biocompatibility) and temperature-responsive polymers in a single macromolecular structure [28–31]. We demonstrate that the PEG-chitosan nanogels can not only respond to the changes in environmental pH over the physiologically important range of 5.0–7.4, but, more importantly, also enable us to remotely modulate the pH-response by external cooling/heating. An unusual feature of the pH-induced swelling behavior of the nanogels is a reentrant phenomenon where the nanogels swell and then re-collapse as the surrounding temperature is varied monotonically. The behavior is similar to the reentrant phenomenon of a binary fluid system [32], which so far is only observed in bulky gels [33,34]. Under such a design, the specific disruptions in the acid/base homeostasis of the local pathological environment (endogenous activation) can provide a biologically regulated release of a model anticancer drug 5-fluorouracil (5-FU), while the external cooling/heating can provide orthogonal stimulus (exogenous activation) for spatiotemporal regulating of the regulator. Thus, PEG-chitosan nanogels serving as drug carriers may not only provide basal chemo-treatment for daily care under the endogenous activation strategy, but also offer both fast-acting (via positive tunability by combining with cryo-treatment) and slow-acting (via negative tunability by combining with thermo-treatment) dosage under exogenous activation strategy, which will enhance our ability to address the complexity of biological systems with remarkable spatiotemporal resolution.

2. Materials and methods

2.1. Materials

Human serum albumin (HSA, 66.4 kDa) was purchased from Tokyo Chemical Industry (TCI), and all other chemical reagents were purchased from Sigma-Aldrich. 2-(2-methoxyethoxy)ethyl methacrylate (MEO_2MA, 95%), oligo(ethylene glycol)methyl ether

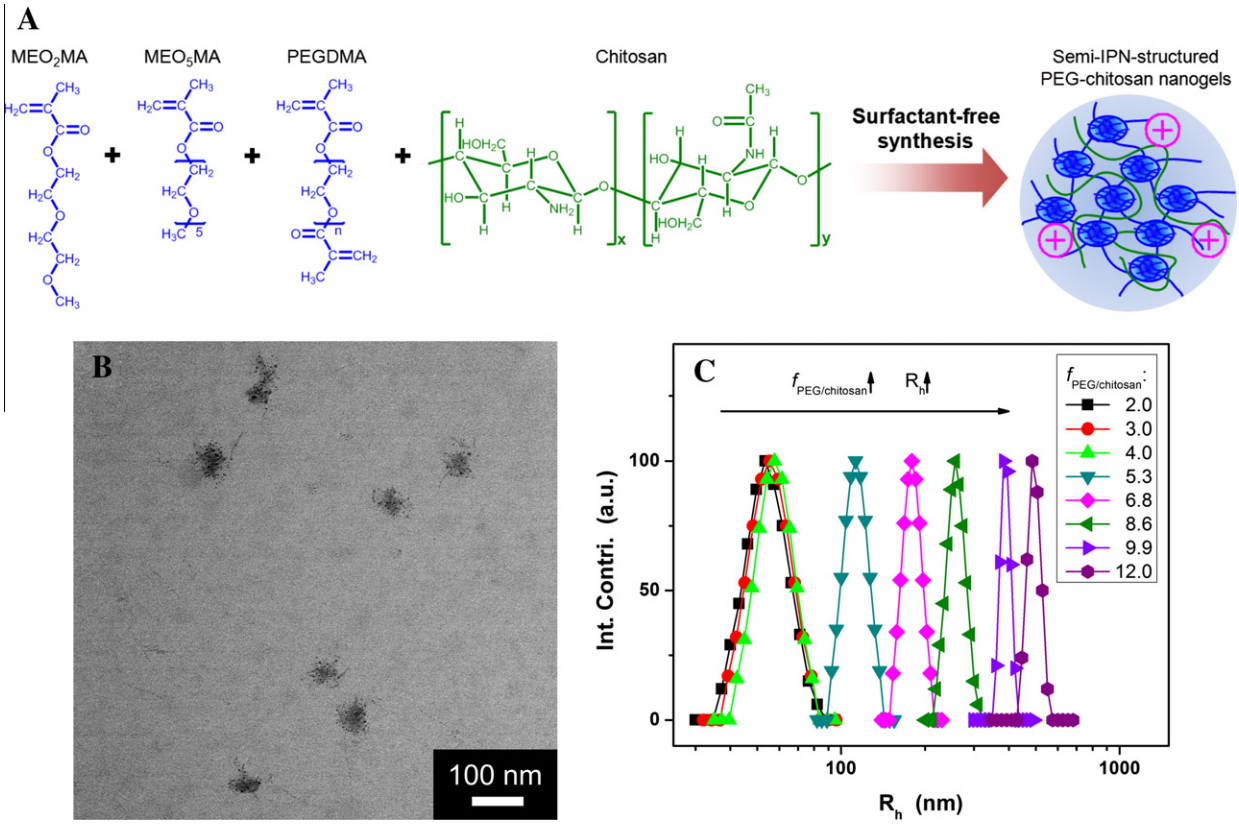

Fig. 1. (A) Schematic illustration of PEG-chitosan nanogels with a semi-IPN structure. (B) TEM images of PEG-chitosan nanogels (MG1). The sample was dried from a dilute dispersion of pH = 7.4 at room temperature. (C) The effect of mole ratio $f_{PEG/chitosan}$ values in synthetic feeding conditions on the average R_h of PEG-chitosan nanogel particles. All measurements were made at a scattering angle $\theta = 45°$, pH = 7.4 and $T = 37.0$ °C.

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