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Highly photostable nanogels for fluorescence-based theranostics

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ABSTRACT

A novel photo-crosslinkable nanogel is prepared from a biodegradable polymer template with intrinsic photoluminescence and high photostability. The fluorescent nanogels display excellent biodegradability and cytocompatibility owed to the facile synthesis scheme involving a solvent- and surfactant-free one-pot reaction, derived entirely from biocompatible monomers citric acid, maleic acid, L-cysteine, and poly(ethylene glycol). The resultant nanogels are less than 200 nm in diameter with a narrow size distribution and monodispersity, and demonstrate long-term structural stability in biological buffer for two weeks. To gauge potential in theranostic applications, the fluorescent nanogels were surface functionalized with biologically active RGD peptides and encapsulated with active anti-cancer drug Doxorubicin, resulting in a pH-responsive controlled drug release in acidic pH resembling tumor environments. The strong fluorescence of the nanogels enabled tracking of targeted drug delivery, showing that drug-loaded nanogels homed into the cytoplasmic regions of prostate cancer cells to significantly induce cell death. These photo-crosslinkable and biodegradable nanogels pose as a strong candidate for theranostic medicine, demonstrating versatile functionalization, high stability in biological buffers, and capacity for real-time fluorescence-based monitoring of targeted drug delivery.

1. Introduction

The advance of biodegradable nanoparticles with increasingly sophisticated functions has made far-reaching impact in biomedical engineering to expand bioimaging and drug delivery systems [1–3]. Among these, soft (hydrogel-based) crosslinked nanoparticles offer unique advantages over other drug delivery systems, such as enhanced in-vivo stability, complex interior networks to incorporate bioactive molecules, tunable size ranging from several micrometers to tens of nanometers, and large surface areas for biofunctionalization [4–6]. Furthermore, the use of synthetic polymers in the preparation of crosslinked soft nanoparticles can provide additional advantages in controlling physical, chemical, and biological properties by tailoring the polymer chain and functionality, while accommodating a wider range of monomers with desirable chemistry [7–9].

Recently the diagnostics and therapeutics of disease such as cancer has evolved remarkably due to the rise of multi-functional soft nanoparticles, enabling imaging within delivery systems [10–14]. Fluorescence-based bioimaging offers particular advantages such as high sensitivity and rapid response kinetics [6]. Among fluorescent probes, organic dyes [15] and quantum dots [16–18] are the most widely used that can be encapsulated within or conjugated to the nanoparticle system. However, organic dyes suffer from rapid photobleaching and poor photochemical stability whereas heavy metals (cadmium and selenide) in quantum dots are toxic to living organisms. Moreover, encapsulation or conjugation of fluorescence probes within nanoparticle systems leads to poor performance issues due to leaching, inhomogeneity, or degradation of the fluorescent moieties. As such, materials with intrinsic fluorescence would have a momentous advantages in terms of simplicity and reliability of bioimaging modalities. Much effort in recent years have been directed towards development of intrinsically fluorescent nanoparticles, leading to innovative materials such as carbon nanoparticles [19], silica nanoparticles [20], and intrinsically fluorescent polymeric nanoparticles [21].

Our research group has had a long-standing interest in the development of such intrinsically fluorescent nanoparticles that

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can be prepared from biodegradable polymers. Recently, we synthesized biodegradable photoluminescent polymers (BPLPs) that can emit strong fluorescence with high quantum yield and strong photostability [22]. These polymers demonstrated significant biomedical value in their capacity for fluorescence-based bioimaging, implantable devices such as tissue engineering scaffolds [23], and drug delivery nanoparticles [24–26]. More importantly, the facile synthesis scheme involved a simple polycondensation reaction between metabolites such as citric acid, an amino acid and a diol without the use of solvents or catalysts, facilitating the development of biologically compatible materials. Although BPLPs can precipitate into nanoparticles in water-organic solvent interfaces, they tends to aggregate due to the low molecular weight of BPLPs, severely limiting their potential as theranostic probes. Herein, we describe a simple and convenient strategy for the preparation of highly photostable crosslinked hydrogel particles (nanogels) based on the BPLP template, derived entirely from biocompatible monomers such as citric acid and maleic acid (metabolites in the Krebs cycle) and L-cysteine (an essential amino acid), as well as poly(ethylene glycol) (PEG, widely used in FDA-approved biomaterials). We first synthesized water soluble BPLPs with free radical linkable double bonds to generate photocrosslinkable biodegradable photoluminescent polymers (PBPLPs). These low molecular weight fluorescent polymer chains were crosslinked into nano-scale hydrogels (nanogels) in the presence of a photoinitiator when exposed to ultra violet (UV) light for a few minutes in an aqueous system. The resultant nanogels exhibited strong photoluminescent properties, excellent biocompatibility and detectable cell labeling. Moreover, surface rich functional groups of these nanogels enabled conjugation of a variety of biologically active molecules. These nanogels can be prepared in biologically relevant buffers and media with excellent stability, while demonstrating complete degradation within two weeks. Anticancer drug (DOX) was also incorporated within these fluorescent nanogels to demonstrate efficacy in tracking intracellular drug delivery.

2. Results and discussion

Our synthetic strategy is unique in that overall toxicity inherited by the prepared polymers and nanogels is made minimal by: (1) careful selection of biocompatible monomers, (2) mild one-pot reaction conditions, and (3) avoidance of organic solvents and toxic catalysts. PBPLP polymers are synthesized by a simple polycondensation reaction between carboxylic groups (from citric acid or maleic acid), amine groups (L-cysteine used herein), and a diol (from PEG) to create a low molecular weight polymer chain containing degradable ester and amide bonds, along with an olefin group susceptible to free radical crosslinking (Fig. 1).

The weighted-average molecular mass as measured by MALDI-MS was found to be 1026 Da with a polydispersity index of 1.21 (Fig. S1). NMR chemical shifts featured CH=CH− groups at 6.4 and 6.8 (a), CH2- at 2.9 (b), 3.5 (c), and 4.2 (d) supporting the formation of the proposed structure in Supplementary Fig. S2. After integrating the area under Peak A (characteristic peak for maleic acid), B (characteristic for citric acid), and D (characteristic for ester bond linked PEG), we further confirmed that the ratio between these monomers (0.47:0.51:0.95) within the polymer chains closely matched the feeding ratio (0.5:0.5:1.0) of the monomers citric acid, maleic acid and PEG respectively. It should be noted that the upfield shift of peak B from 2.68 (as in pure citric acid) to 2.9, formation of a new peak at 6.8 (a) compared to pure maleic acid and formation of new peak at 4.2 (d) as compared to pure PEG molecules further supported that polycondensation occurred between carboxylic acid and hydroxyl groups to create PBPLP polymer chains.

To prepare PBPLP nanogels, we dissolved low molecular weight PBPLP polymers in water with low molar ratios of acrylic acid (as crosslinker) and AMPAD (as photo initiator), subjecting them to 395 nm UV light for 15 min, while once again avoiding the use of toxic surfactants and solvents (Scheme 1). The formulated nanogels possessed an average hydrodynamic diameter of 119 ± 47 nm with a mode of 78 nm, with 90% of the nanogels within 185 nm without any evidence of aggregation even at higher concentrations (14.97 × 108 particles/ml) according to the nanogel distribution graph (Fig. 2A). TEM micrographs of PBPLP nanogels revealed spherical particles with good monodispersity (Fig. 2B).

Next, we suspended the nanogels in 0.1 M PBS (pH 7.4) for two weeks to study long-term stability. The nanogels were highly stable during the two weeks, well-maintaining structural integrity with a hydrodynamic diameter within 150 nm (Fig. 2C), after no

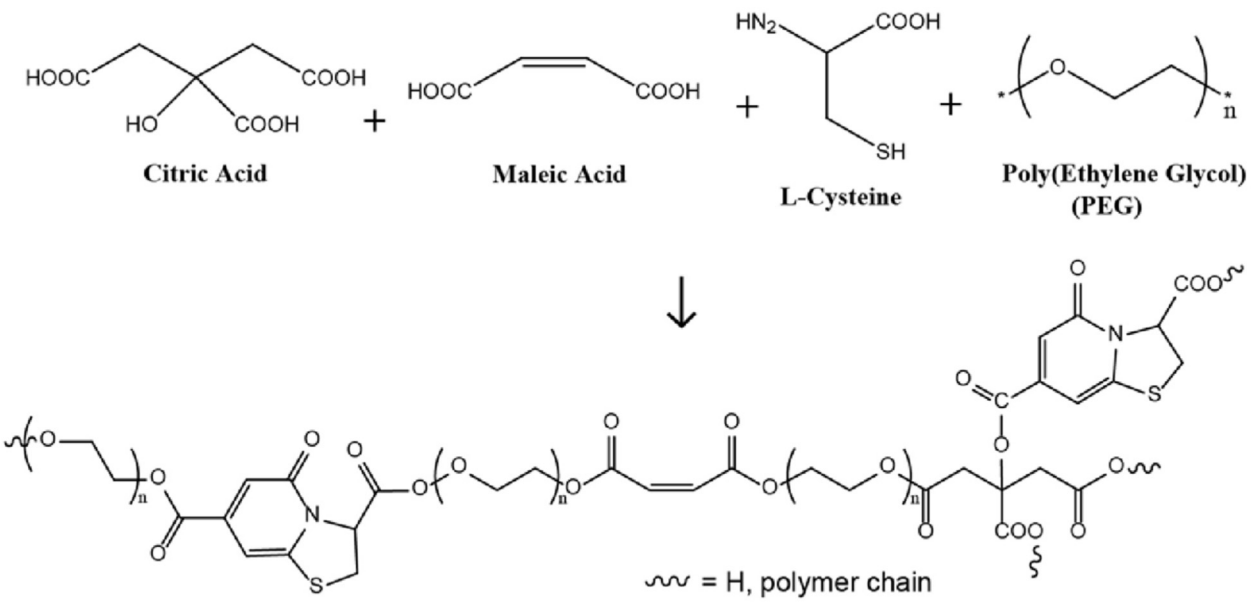

Fig. 1. Monomers used in the synthesis of PBPLP polymer.

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