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Dual responsive nanogels for intracellular doxorubicin delivery

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ABSTRACT

Nanosized polymeric delivery systems that encapsulate drug molecules and release them in response to a specific intracellular stimulus are of promising interest for cancer therapy. Here, we demonstrated a simple and fast synthetic protocol of redox-responsive nanogels with high drug encapsulation efficiency and stability. The prepared nanogels displayed narrow size distributions and versatility of surface modification. The polymer precursor of these nanogels is based on a random copolymer that contains oligoethyleneglycol (OEG) and pyridyldisulfide (PDS) units as side-chain functionalities. The nanogels were prepared through a lock-in strategy in aqueous media via self cross-linking of PDS groups. By changing polymer concentration, we could control the size of nanogels in range of 80–115 nm. The formed nanogels presented high doxorubicin (DOX) encapsulation efficiency (70% (w/w)) and displayed pH and redox-controlled drug release triggered by conditions mimicking the reducible intracellular environment. The nanogels displayed an excellent cytocompatibility and were effectively endocytosed by A2780CP ovarian cancer cells, which make them promising nanomaterials for the efficient intracellular delivery of anticancer drugs.

1. Introduction

Nanosized polymeric drug delivery systems are developed to improve the therapeutic efficacy and to reduce unwanted side effects of anticancer drugs. These delivery systems accumulate in the tumor after intravenous injection via the enhanced permeability and retention (EPR) effect (Bertrand et al., 2014; Brannon-Peppas and Blanchette, 2004; Maeda, 2010; Maeda et al., 2000). However, few of the polymeric delivery systems have achieved satisfactory outcomes, which is very often due to a poor intracellular trafficking process and/or inefficient drug release inside targeted cells (Breunig et al., 2008; Nori and Kopecek, 2005). To that end, design and synthesis of nanosized drug containing systems with capability of intracellular responsiveness are attractive for cancer therapy (Allen and Cullis, 2004; Peer et al., 2007; Ryu et al., 2010; Savariar et al., 2006). Among nanosized polymeric drug delivery systems, micelles are promising candidates mainly for delivery of hydrophobic drugs (Savić et al., 2003). However, non cross-linked micelles have a few shortcomings including inherent stability issues and premature release of the cargo from their core at undesirable locations (Bae and Yin, 2008; Chen et al., 2008). In order to overcome these issues, researchers developed, chemically cross-linked water-soluble polymer nanoparticles, nanogels (NGs). Compared to micelles, NGs show high aqueous dispersability and stability, well-defined structure, high encapsulation capacity and suitability for biomacromolecules delivery (Chacko et al., 2012; Kabanov and Vinogradov, 2009; Li et al., 2015; Oh et al., 2008).

Although NGs constitute a promising scaffold in therapeutic delivery applications, they face certain complications, as they are prepared by microemulsion or inverse microemulsion methods (Oh et al., 2008). Microemulsion methods, which involve oil-in water emulsion, utilize lipophilic monomers to produce the NGs, which are thus generally water insoluble. When a water-soluble polymer nanoparticle is desired, inverse microemulsion based synthesis is the preferable method. Although in the inverse microemulsion method, the continuous phase is a lipophilic solvent and therefore cannot be used to encapsulate hydrophobic drugs during NGs formation. To that end, it is needed to invent a facile method that allows for the design and synthesis of NGs under non-emulsion conditions with high lipophilic encapsulation capabilities, and high stability. Furthermore, it would be much more promising if NGs have stimuli sensitive behavior because this interesting feature enables them to be attractive candidates as controlled drug delivery systems.

To obtain NGs with mentioned characteristics, one should incorporate a functional group that specifically responds to a

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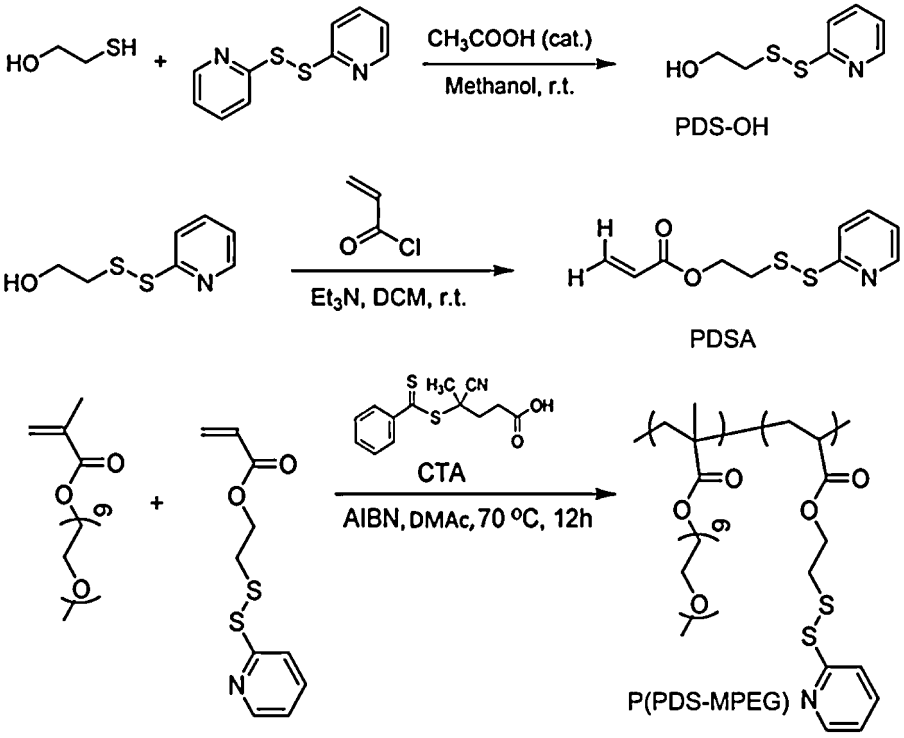
biologically relevant stimulus and the NGs should be synthesized in the aqueous phase from a water-soluble temperature responsive precursor copolymer. Temperature responsive segments in the polymer design can transit their hydrophilic characteristics to hydrophobic upon heating above their lower critical solution temperature (LCST) and cause the formation of nano-self-assembled structures, which can be turned into NGs by cross linking. As a stimuli-responsive functional group, functional monomers containing pyridyldisulfide (PDS) with capability of formation of disulfide bonds are promising, due to the fact that these bonds are susceptible to biochemical reductants such as glutathione (GSH), thioredoxin, and peroxiredoxin (Cheng et al., 2011; Meng et al., 2009). Therefore, disulfide bonds can be easily degraded in the reducing intracellular environment, while remaining stable in the extracellular space. This phenomenon is an interesting approach to design redox sensitive nanosystems for intracellular drug delivery as reported by different groups (Chen et al., 2012; Remant Bahadur et al., 2012; Pan et al., 2012; Wang et al., 2011). In some studies, the PDS side chain functionality was used as a handle to incorporate thiol-based functional groups into polymers (Ghosh et al., 2006; Jia et al., 2009; Wong et al., 2008; Zugates et al., 2006). Using the reactivity of the PDS functionality, one can obtain cross-linking in polymer chains and prepare NGs. In addition, as such an NG has hydrophobic feature, it can be used for encapsulation of hydrophobic guest molecules.

Among temperature responsive polymers, poly(*N*-isopropylacrylamide) (PNIPAM) is perhaps the mostly studied thermosensitive polymer and as such the majority of thermosensitive NGs studied are based on PNIPAM (Ramos et al., 2012), but recently other types of polymers are increasingly being investigated for their thermoresponsive behavior as PNIPAM alternatives (Hoogenboom, 2009; Lutz, 2008; Vancoillie et al., 2014). Hence, polymers bearing oligo ethylene glycol (OEG) side chains have attracted much interest as thermosensitive polymers because of having excellent biocompatibility, sharpness of responsiveness, antifouling properties below LCST and bio-inertness and more importantly, a much more uniform thermal profile during heating and cooling cycles in comparison with PNIPAM (Lutz et al., 2007, 2006). In other words, OEG based polymers can combine both the features of PEG and PNIPAM in a single macromolecule. As a result, they are promising candidates as temperature responsive segment.

Here, we took the advantages of both PDS and OEGMA groups for designing stimuli responsive NGs. To the best of our knowledge, there is only one study used random copolymer consist of OEG methacrylate and PDS-derived methacrylate for nanogel preparation, which has some drawbacks (Ryu et al., 2010). The hydrophobic feature of used copolymer has effect on their thermoresponsivity and water solubility. Improper design of copolymer composition caused to formation of nanogels with broad PDI. In addition to that, prepared NGs did not show high encapsulation efficiency and complete release due to incomplete disintegration in aqueous media. To solve this problem, a random copolymer was designed in such a way to be soluble at room temperature and form mono sized nano-self assembled structure upon heating above their LCST. Hence, this work sought to fabricate monosized NGs with a simple and fast synthetic protocol with capability of high encapsulation efficiency, high stabilities and redox responsiveness for intracellular doxorubicin delivery. In this contribution, the NGs were prepared, using a well-designed random copolymer containing OEG and PDS units, through the disulfide cross-linking of PDS groups in aqueous media. The key feature in our study was the utility of an amphiphilic random copolymer, where the reactive lipophilic functional groups, PDS, were utilized for cross-linking, drug encapsulation, and inserting redox sensitive bond into NGs and the hydrophilic OEG groups induced temperature responsive characteristics and charge-neutral functional groups which made it possible to prepare NGs in aqueous solution. Accordingly, the synthesized polymer and NGs were characterized in vitro with different techniques and the effectiveness of prepared NGs as promising intracellular anticancer delivery system was confirmed by release, toxicity, anticancer activity, and uptake studies.



Scheme 1. Schematic representation of synthesis of side chain reactive PDS-MPEG random copolymer.