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Encapsulating magnetic and fluorescent mesoporous silica into thermosensitive chitosan microspheres for cell imaging and controlled drug release *in vitro*

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ABSTRACT

In this study, for the first time, multifunctional inorganic/organic core/shell hybrid microspheres consisted of Fe_3O_4 nanoparticles/CdTe quantum dots dual-embedded mesoporous silica nanocomposites (MQ-MSN) as cores and P(*N*-isopropylacrylamide)-*graft*-Chitosan microgels (PNIPAM-*g*-CS) as shells were prepared by copolymerization of NIPAM and CS in the presence of MQ-MSN. The preparation of microspheres (*i.e.*, MQ-MSN/PNIPAM-*g*-CS) included three stages. First, Fe_3O_4/CdTe nanocomposites (MQ NCs) were prepared by self-assembly of electrostatic adsorption. Second, MQ NCs were encapsulated into silica spheres by modified Stöber method to obtain MQ-MSN. Third, NIPAM monomers were initiated to fabricate PNIPAM networks with MQ-MSN distributed below the lower critical solution temperature (LCST) of PNIPAM, and then PNIPAM reacted with CS to form PNIPAM-*g*-CS copolymers above the LCST, meanwhile the PNIPAM networks collapsed to form microspheres, resulting in the MQ-MSN encapsulated into microspheres. The microspheres were systematically characterized, displaying perfect magnetic/fluorescent properties and thermo-sensitivity. HepG2 cancer cells treated with the microspheres revealed bright fluorescence imaging. Both the efficiency and capacity of Adriamycin (ADM) loaded into the microspheres were gradually increased with ADM concentration increasing. The ADM cumulative release *in vitro* from ADM-loaded microspheres was significant at a higher temperature (or a lower pH). The released ADM still maintained high anticancer activity, and the blank microsphere carriers hardly produced toxicity to HepG2 cells. Hence, the multifunctional microspheres exhibited a promising application especially as thermo/pH-sensitive drug carriers for *in vivo* therapy.

1. Introduction

During the past decade, great efforts had been devoted to synthesizing stimuli-sensitive microspheres that could respond to various external stimuli, which made the microspheres especially suitable for applications in biological and biomedical fields [1–11]. In particular, thermosensitive poly(*N*-isopropylacrylamide) (PNIPAM) has attracted much attention and was widely investigated because PNIPAM has a lower critical solution temperature (LCST) of ~32–34 °C in water. When external temperature exceeds the LCST, PNIPAM networks will suffer from an abrupt collapse to form hydrophobic microspheres, a coil-globule volume transition [12,13]. Since firstly reported in 1986 [14], PNIPAM as a promising drug delivery system (DDS) has received considerable attention because its transition temperature can be modulated close to body temperature. A key challenge to use PNIPAM is to modulate its LCST around body temperature [15–19]. To date, several attempts have been made to modulate the LCST of PNIPAM preferably up to 42–43 °C for hyperthermia treatment of cancer [20]. Yuan et al. reported that the copolymerization of *N*,*N*′-dimethylacrylamide (DMA) and NIPAM induced a shift in LCST from 32 to 38 °C. The resultant PNIPAM is not biodegradable, and requires to be modified or conjugated with other polymers to develop biodegradable DDS [12,21]. As known, chitosan (CS), derived from *N*-deacetylation of chitin and extracted from crustacean shells, is a natural polycationic polymer owning biocompatibility, low-toxicity and pH-sensitivity, as well as high affinity for cell membranes [21–23]. In hydrosoluble state, the positively charged CS interacts well with the negatively charged PNIPAM, which enables forming PNIPAM/CS hydrogels that can serve as thermo/pH-sensitive DDS. Rejinold et al. prepared CS-*g*-PNIPAM polymers by ionic cross-linking method. The polymer exhibited a LCST of 38 °C, and was developed toward *in vitro* drug release [24]. Gong et al. synthesized PNIPAM/CS microspheres *via* microemulsion, and investigated pH/thermo-dependent drug release from the microspheres [25].

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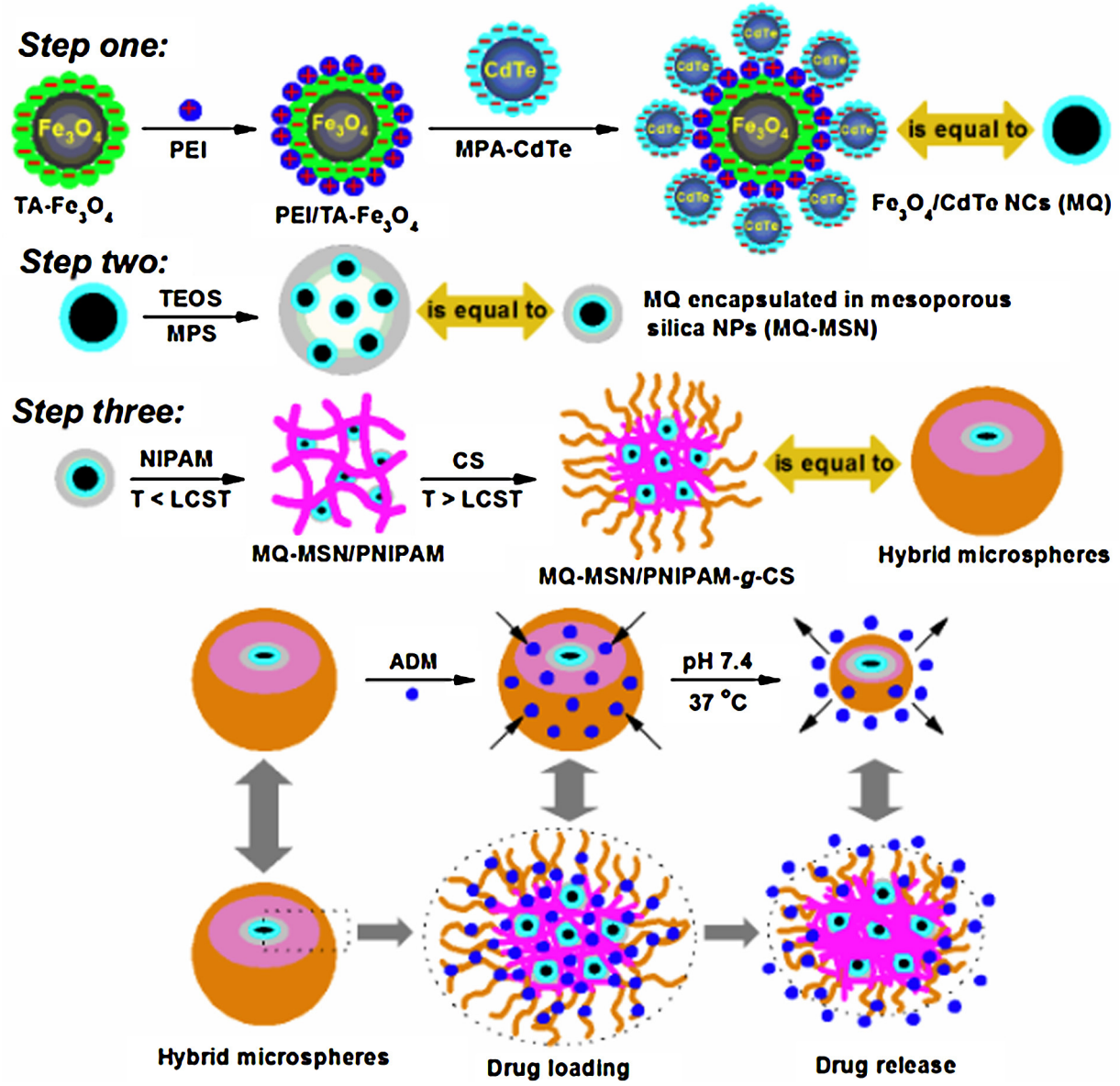

Scheme 1. Schematic illustration of the preparation, ADM loading and release of MQ-MSN/PNIPAM-*g*-CS hybrid microspheres.

Recently, the development of magnetic drug carriers with fluorescent marker (organic dye or quantum dots, QDs) has become a favorite topic for targeted and site-specific drug delivery with reduced side effects [8]. In this regard, superparamagnetic nanoparticles (SPMNPs) (*e.g.*, Fe_3O_4) with high magnetization and good biocompatibility have attracted particular attention as high-quality DDS [20,26,27]. To avoid being rapidly cleared by reticuloendothelial system or macrophages before arriving at desired sites, SPMNPs should be modified by functionalized groups for applications in DDS. The preferred DDS is characterized by functionalized SPMNPs as cores and biodegradable polymers as shells. Thus, anticancer drugs are covered by polymer shells during delivery process, and the possible side effects of drugs can be minimized [28]. For instance, Fe_3O_4/PNIPAM/CS hybrid microspheres were fabricated *via* emulsion polymerization of CS and NIPAM in the presence of oleic acid modified Fe_3O_4 NPs [22]. Fe_3O_4 NPs encapsulated nanohydrogels were synthesized by free radical polymerization of NIPAM in the presence of CS and Fe_3O_4. The encapsulation of Fe_3O_4 caused an increase in LCST near 42 °C [29]. To add the actions of cell tracking, migration imaging and drug anchoring *in vivo*, QDs were introduced into DDS due to preferred photoluminescence (PL) properties for biological applications, compared to fluorescent dyes [30–34]. Researchers have attempted to achieve magnetic QDs (MQ) and MQ-embedded microspheres. Carboxymethylchitosan-based folate/Fe_3O_4/CdTe microspheres were prepared for targeted drug delivery and cell imaging, exhibiting high drug loading efficiency, low cytotoxicity and favorable cell compatibility [35]. Guo et al. developed silica-coated Fe_3O_4@SiO_2@CdTe microspheres covered with PNIPAM outer shells [33]. Chang et al. prepared magnetic mesoporous silica-embedded P(NIPAM-*co*-methacrylic acid) microspheres for controlled drug release [36].

Currently, mesoporous silica NPs (MSN) as promising colloidal drug carriers have been highlighted [37,38]. In view of their prominent biocompatibility, MNS may be applied in controlled drug release that can match actual physiological needs at a proper site and time. However, to the best of our knowledge, until now thermosensitive chitosan (PNIPAM-CS) coated Fe_3O_4/QDs dual-embedded MSN have been no reported. The novel PNIPAM/CS/Fe_3O_4/QDs system, combining thermo/pH-sensitivity and magnetic/fluorescent/biocompatible/low-toxic properties into