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Reducible polyamidoamine-magnetic iron oxide self-assembled nanoparticles for doxorubicin delivery

Jun Chen [a,b,c,d,h,1], Min Shi [e,1], Pengmin Liu [e], Alex Ko [f], Wen Zhong [g], WangJun Liao [e,**], Malcolm M.Q. Xing [a,b,c,d,*]

[a] School of Basic Medical Science, Southern Medical University, Guangzhou 510515, China
[b] Department of Mechanical Engineering, University of Manitoba, Winnipeg MB R3T 2N2, Canada
[c] Department of Biochemistry and Medical Genetics, University of Manitoba, Winnipeg MB R3T 2N2, Canada
[d] Manitoba Institute of Child Health, Canada
[e] Department of Oncology, Nanfang Hospital, Southern Medical University, Guangzhou 510515, China
[f] National Research Council Canada, Medical Device Portfolio, Winnipeg, Manitoba, Canada
[g] Department of Medical Microbiology, University of Manitoba, Canada
[h] CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

We report a reducible copolymer self-assembled with superparamagnetic iron oxide nanoparticles (SPIONs) to deliver doxorubicin (DOX) for cancer therapy. The copolymer of reducible polyamidoamine (rPAA) with poly(ethylene glycol)(PEG)/dodecyl amine graft was synthesized by Michael addition. rPAA@SPIONs were formed by the alkyl grafts of reducible copolymers intercalated with the oleic acid layer capped on the surface of magnetite nanocrystals. The intercalating area formed a reservoir for hydrophobic anti-cancer drug (DOX), whilst the PEG moiety in the copolymers helped the nanoparticle well-dispersible in aqueous solution. We employed two-photon excited fluorescence (TPEF) and coherent anti-Stokes Raman (CARS) to investigate drug delivery in intra-cellular structures of live cells, and used Vivaview® technique to show real-time inhibition efficacy of nanoparticles in live cells. rPAA@SPIONs present efficiently drug loading with reducible responsibility in vitro tests. Finally, rPAA@SPIONs were tested in mice with xenograft MDA-MB-231 breast tumor though i.v. injection and inhibited tumor growth efficiently. MRI was used to monitor nanoparticles aggregation in tumor site. Histology and Prussian blue on kidney, liver, and heart in mice indicated that DOX/rPAA@SPIONs showed no significant toxicity for mice organs after 24 days treatment.

1. Introduction

Magnetic iron oxide (IO) nanoparticles are extensively studied as a promising theranostic candidate for magnetically targeted drug delivery and molecular magnetic resonance imaging (MRI) diagnosis [1–4]. Monodisperse and highly crystalline IO nanoparticles can be produced using a high-temperature decomposition method [5–9]. Later on, Namiki and coworkers used a more mild method, modified peptization technique to generate the oleic-acid coated superparamagnetic IO nanoparticles (OA@SPIONs) [10]. However, these nanoparticles have limits in the stability because of hydrophobic coating. To address issue, Qin and coworkers encapsulated the SPOINs into the Pluronic F127 (PF127) by the hydrophobic–hydrophobic mechanism [11]. PF127 block copolymer is a suitable candidate of surface modification which has been broadly used in experimental medicine and pharmaceutical sciences. This composition exhibits highly coating efficiency and stable dispensability in aqueous solutions for SPIONs. In long-term in *vivo* efficacy, amphiphilic copolymers coated oleic acid (OA) @SPIONs was also studied. For example, poly(D, L-lactic-co-glycolic acid) nanoparticles (size >100 nm) containing multiple oleic acid-coated IO nanocrystals (each IO size ≈ 10 nm) has been tested [12]. These micellar nanoparticles were reported with both good biodegradability and MRI contrast effect but not for a controlled release or environmental response. To address this issue, a thermosensitive

* Corresponding author. Department of Mechanical Engineering, University of Manitoba, Winnipeg MB R3T 2N2, Canada. Fax: +1 204 2757507.
** Corresponding author. Fax: +86 020 62787731.
E-mail addresses: nfyyliaowj@163.com (W. Liao), malcolm.xing@umanitoba.ca, xing@cc.umanitoba.ca (M.M.Q. Xing).
[1] Equal contribution.

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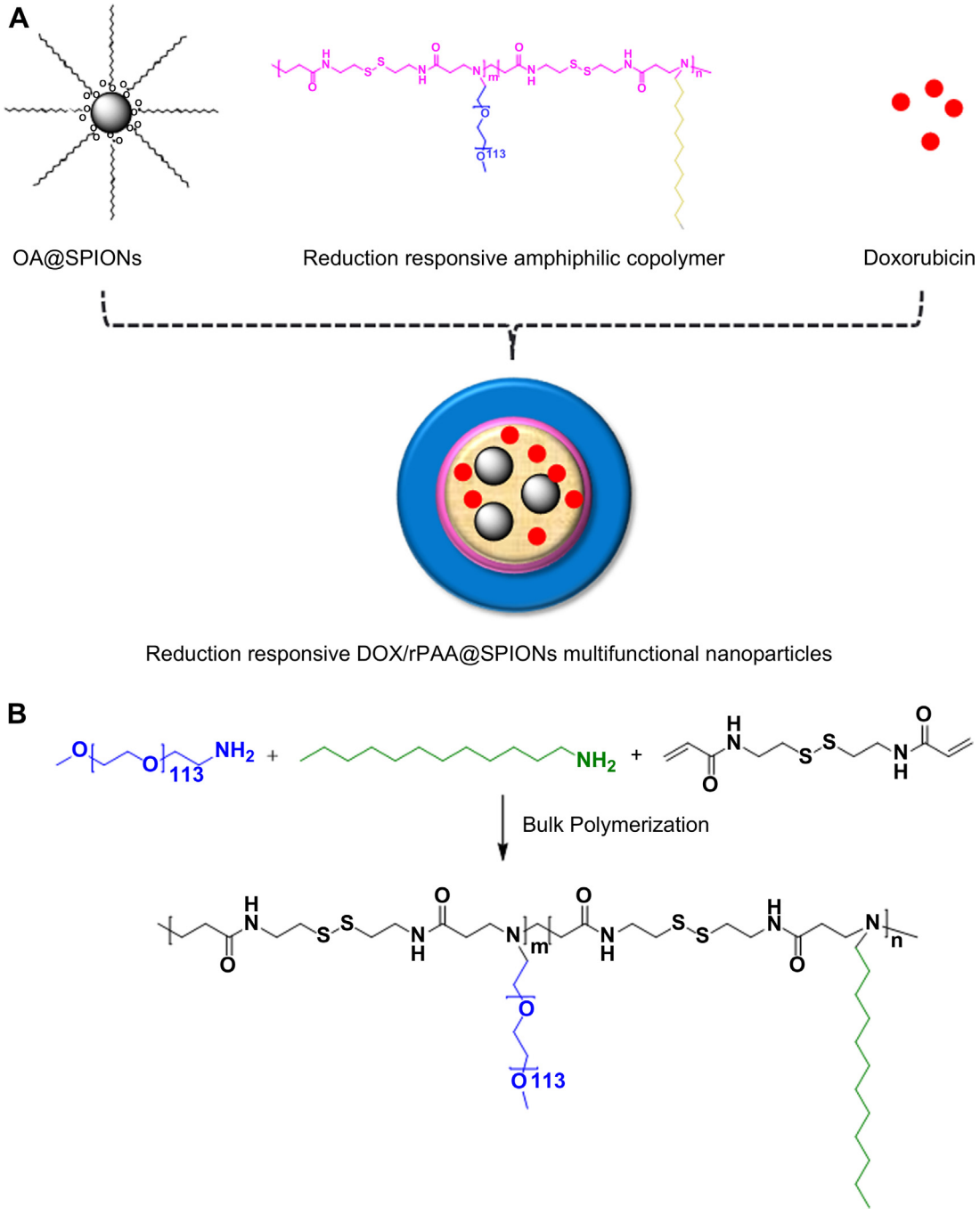

Fig. 1. A. The schematic illustration of the architecture of doxorubicin loaded reducible copolymer coated superparamagnetic iron oxide nanoparticles; B. The synthesis route of reducible polyamidoamine graft poly(ethylene glycol)/dodecyl amine copolymers (rPAA); C. The ^{1}H NMR spectrum of rPAA copolymer.

poly(maleic anhydride-*alt*-octadecene) –*graft* -PNIPAAm (PMAO-*g*-PNIPAAm) copolymer was exploited to coat on the OA@SPIONs [13]. As expected, the modified SPIONs showed a sharp transition upon change of temperature. This study provided a very promising potential strategy to build up a "smart" nanocarrier for drug delivery but regretful, the authors didn't present the work for drug delivery. So far, although there are numerous magnetic nanoparticles fabricated as drug carriers, only a few were developed as both MRI contrast agent and stimuli-responsive drug delivery system in *vitro* and in *vivo*.

Cancer is a leading cause of death and its diversity always evades monotherapy. Environmental-sensitive nanomaterials have been emerging interests due to their great potential in cancer therapy. Tremendous development and investigation have been conducted to design nanoparticles sensitive to external cues like redox potential, temperature, pH value and specific analytes [13–24] for drug release in tumor. For example, reducing agents concentration is about 1000 times in the various subcellular organelles in cytoplasm (millimolar) than the extracellular environment (micromolar) [25–27]. Disulfide bonds are reductively degraded in the reducing intra-cellular environment easily, while remaining un-cleavable in a predominantly oxidizing extracellular space [28–30]. The intra-cellular cleavage of disulfide bonds in nanoparticles is mostly mediated by thiol/disulfide exchange