Biomaterials 114 (2017) 23–33

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# A superparamagnetic polymersome with extremely high $T_2$ relaxivity for MRI and cancer-targeted drug delivery

Qiuming Liu [a, b, 1], Liwen Song [a, 1], Shuai Chen [b], Jingyi Gao [b], Peiyu Zhao [a], Jianzhong Du [a, b, *]

[a] Shanghai Tenth People's Hospital, Tongji University School of Medicine, 301 Middle Yanchang Road, Shanghai 200072, China
[b] Department of Polymeric Materials, School of Materials Science and Engineering, Tongji University, 4800 Caoan Road, Shanghai 201804, China

ARTICLE INFO

*Article history:*
Received 10 July 2016
Received in revised form
12 October 2016
Accepted 15 October 2016
Available online 15 October 2016

*Keywords:*
MRI
Superparamagnetic iron oxide nanoparticles
Polymersome
Polymer vesicle
Antitumor drug delivery
Self-assembly

ABSTRACT

Improving the relaxivity of magnetic resonance imaging (MRI) contrast agents is an important challenge for cancer theranostics. Herein we report the design, synthesis, characterization, theoretical analysis and *in vivo* tests of a superparamagnetic polymersome as a new MRI contrast agent with extremely high $T_2$ relaxivity (611.6 $mM^{-1}s^{-1}$). First, a noncytotoxic cancer-targeting polymersome is synthesized based on a biodegradable diblock copolymer, folic acid-poly(L-glutamic acid)-*block*-poly($\varepsilon$-caprolactone) [FA-PGA-*b*-PCL]. Then, ultra-small superparamagnetic iron oxide nanoparticles (SPIONs) are *in situ* generated in the hydrophilic PGA coronas of polymersomes to afford magnetic polymersomes. The *in vivo* MRI assay revealed prominent negative contrast enhancement of magnetic polymersomes at a very low Fe dose of 0.011 mmol/kg. Moreover, this cancer-targeting magnetic polymersome can effectively encapsulate and deliver anticancer drug to inhibit the tumor growth, demonstrating promising theranostic applications in biomedicine.



## 1. Introduction

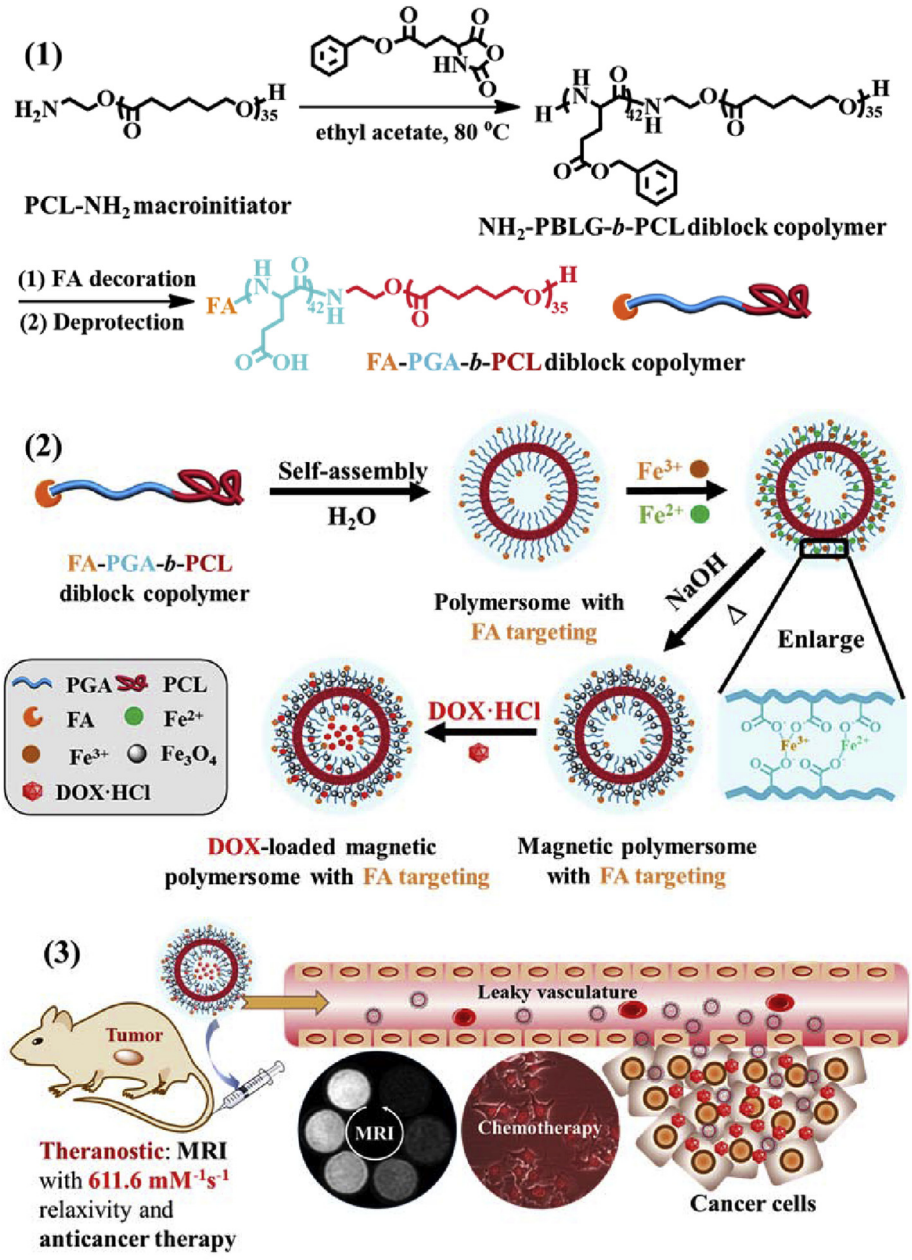
MRI is one of the most powerful and non-invasive clinical imaging modalities with high spatial resolution. Its sensitivity can be significantly improved in the presence of contrast agents [1–3]. The efficiency of positive (*e.g.*, $[Gd(DTPA)]^{2-}$ complex) and negative (*e.g.*, SPIONs) MRI contrast agents can be improved by shorten the longitudinal ($T_1$) or transverse ($T_2$) relaxation times of the proton spins of water in tissues [4,5]. However, gadolinium-based contrast agents may lead to nephrogenic systemic fibrosis [6]. In contrast, SPIONs-based MRI contrasts are less toxic than the gadolinium-based analogues [7,8]. In the past decades, various SPIONs-based $T_2$ MRI contrast agents were studied [9–11]. Their relaxivity was influenced by the nature and the size of the ultra-small SPIONs [12,13], clustering effect (several SPIONs per contrast agent) [14,15], non-magnetic shell [16,17], hydrophobic membrane [18,19], and water permeability, etc. [20,21].

In the past decades, polymeric nanostructures self-assembled from block copolymers were used to stabilize SPIONs by *ex situ* incorporating into polymeric nanostructures [22–24]. For example, Park et al. reported size-controlled self-assembly of magneto-polymersomes through the cooperative self-assembly of iron oxide nanoparticles and amphiphilic polymers [25]. They also developed another superparamagnetic polymersome with a $T_2$ relaxivity of 555 ± 24 $mM^{-1}s^{-1}$ [26]. In 2013, Riffle et al. reported magnetic block ionomer clusters with $T_2$ relaxivities ranging from 190 to 604 $mM^{-1}s^{-1}$ [21]. However, those magnetic nanoparticles have not been used as theranostic agents for biomedical applications. It is still an important challenge to improve the $T_2$ relaxivity for minimizing the dosage of MRI cotrast agent [4,27,28].

Recently, polymersomes (also called polymer vesicles) have attracted much attention due to their wide potential applications [29–33]. Herein, we develop a novel approach to fabricate cancer-targeting SPIONs with an extremely high $T_2$ relaxivity (611.6 $mM^{-1}s^{-1}$) [26] based on *in situ* chemical precipitation in the hydrophilic coronas of a biodegradable polymersome. As shown in Scheme 1 and Fig. S1, the amphiphilic FA-PGA-*b*-PCL diblock

* Corresponding author. Department of Polymeric Materials, School of Materials Science and Engineering, Tongji University, 4800 Caoan Road, Shanghai 201804, China.
*E-mail address:* jzdu@tongji.edu.cn (J. Du).
[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.biomaterials.2016.10.027
0142-9612/




**Scheme 1.** Illustration of multifunctional magnetic polymersomes for ultrasensitive $T_2$ MRI and cancer-targeted drug delivery. (1) Synthetic route to FA-PGA-*b*-PCL diblock copolymer and (2) DOX-loaded magnetic polymersome with folic acid (FA) targeting unit. (3) Schematic demonstration of the processes of $T_2$-weighted MRI and chemotherapy after injection of the DOX-loaded magnetic polymersome with FA targeting unit into the nude mice bearing tumor *via* tail vein. Blue: hydrophilic and biodegradable peptide PGA. Red: biocompatible and biodegradable PCL. Orange: tumor targeting FA. Brown: ferric ion, $Fe^{3+}$. Green: ferrous ion, $Fe^{2+}$. Black: SPIONs, $Fe_3O_4$. Pink: doxorubicin hydrochloride (DOX·HCl).

copolymer was synthesized by ring-opening polymerization of glutamic acid using PCL-$NH_2$ as a macroinitiator. Then the copolymer was self-assembled into polymersomes in aqueous solution. The negatively charged coronas of polymersomes can interact with the positively charged ferric ($Fe^{3+}$) and ferrous ($Fe^{2+}$) ions. As a result, ultra-small $Fe_3O_4$ nano-clusters with prominent transverse relaxivity formed in the coronas of polymersomes upon adding aqueous NaOH solution. Furthermore, the anticancer drug DOX·HCl can be encapsulated into the cavity of the polymersome and adsorbed on the coronas, then delivered into cancer cells. Moreover, cancer-targeting FA is anchored on the polymersome for better anticancer drug delivery and MRI.

## 2. Materials and methods

### 2.1. Materials

γ-Benzyl-L-glutamate, triphosgene and hydrogen bromide (33 wt% in acetic acid) were purchased from Shanghai Hanhong Chemical Co., Ltd. ε-Caprolactone (Aldrich) was dried azeotropically using anhydrous toluene prior to use. *N*-(*tert*-Butoxycarbonyl) 2-aminoethanol, stannous 2-ethylhexanoate ($Sn(Oct)_2$), trifluoroacetic acid (TFA), α-pinene and folic acid (FA) were obtained from Aladdin and used as received. O-(7-Azabenzotriazole-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU), diisopropylethylamine (DIPEA), 2,2′-(ethylenedioxy)bis(ethylamine)