Biomaterials 69 (2015) 184–190

Contents lists available at ScienceDirect

Biomaterials

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

Hydrophilic packaging of iron oxide nanoclusters for highly sensitive imaging

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ARTICLE INFO

Article history:
Received 15 April 2015
Received in revised form
27 July 2015
Accepted 31 July 2015
Available online 4 August 2015

Keywords:
Nanocluster
Molecular architecture
Contrast agent
Diagnostic imaging
Polyglycerol

ABSTRACT

Superparamagnetic iron oxide nanoparticles (SPIONs) are used as imaging probes to provide contrast in magnetic resonance images. Successful use of SPIONs in targeted applications greatly depends on their ability to generate contrast, even at low levels of accumulation, in the tissue of interest. In the present study, we report that SPION nanoclusters packaged to a controlled size by a hyperbranched polyglycerol (HPG) can target tissue defects and have a high relaxivity of 719 mM^{-1} s^{-1}, which was close to their theoretical maximal limit. The resulting nanoclusters were able to identify regions of defective vasculature in an ischemic murine hindlimb using MRI with iron doses that were 5–10 fold lower than those typically used in preclinical studies. Such high relaxivity was attributed to the molecular architecture of HPG, which mimics that of the water retentive polysaccharide, glycogen. The results of this study will be broadly useful in sensitive imaging applications.

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1. Introduction

Magnetic resonance imaging (MRI) generates high-resolution images non-invasively and therefore is used for diagnosis of various tissue defects [1,2] as well as evaluation of fluid flows within engineered materials and devices [3–5]. The diagnostic capability of MRI has been greatly enhanced with the introduction of superparamagnetic iron oxide nanoparticles (SPIONs), which can provide negative contrast against surrounding tissues. To further enhance their utility, SPIONs that have increased relaxivity while localizing at sites of interest would be advantageous for improving the capabilities of MRI. An attractive method to accomplish this is to tailor particle size within a range for which relaxivity is maximized, known as the static dephasing regime (SDR) [6]. However, SPIONs with diameters approaching such an optimal size often become permanently magnetic [7], resulting in uncontrolled aggregation that diminishes relaxivity and substantially reduces the SPION's ability to reach target sites. Assembling SPIONs in the form of clusters has emerged as a promising strategy to yield a desired size of metallic core while maintaining superparamagnetism and colloidal stability [8–14]. A limitation of common approaches, however, lies in the SPION coating material used to induce clustering, as such materials may limit penetration of water near the metallic core or reduce hydrophilic interactions. Such factors are intrinsically detrimental since the effectiveness of an MR contrast agent is highly dependent on its ability to interact with surrounding water [15].

In this study, we hypothesized that the globular nature of a hydrophilic, hyperbranched polymer would allow for maximal MR relaxivity of clustered SPIONs to improve the diagnostic capability of *in vivo* imaging of ischemic tissue. To test this hypothesis, SPIONs

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.056
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were coated by a hyperbranched polyglycerol substituted with a varying number of octadecyl chains (HPG-g-C_{18}) to form the nanoclusters (Fig. 1a). Hyperbranched polyglycerol was adopted to mimic the structure of glycogen, a natural, hyperbranched biopolymer that is able to hold 2–3 times its weight in water [16]. The critical role of the polyglycerol molecular architecture in enhancing relaxivity of SPION clusters was addressed using the analogous linear polyglycerol substituted with octadecyl chains as a control. The ability of the resultant ultrasensitive nanocluster to identify ischemic tissue vascularized with leaky blood vessels was then evaluated in a murine model of hindlimb ischemia [17].

2. Materials and methods

All materials were purchased from Sigma–Aldrich unless otherwise noted.

2.1. General polyglycerol characterization methods

Mass spectral analysis was performed using ESI on a Waters Micromass Q-Tof spectrometer or MALDI-TOF on an Applied Biosystems Voyager-DE STR spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian U400, UI400, U500 or VXR500 spectrometer. Additionally, the molecular weights of HPGs were evaluated by gel permeation chromatography (GPC, Waters Breeze 2) with a Styragel HT column. 20 mM LiBr in *N,N*-dimethylformamide (DMF) was used as an eluent. Values were based on calibration against polyethylene glycol (PEG) standards.

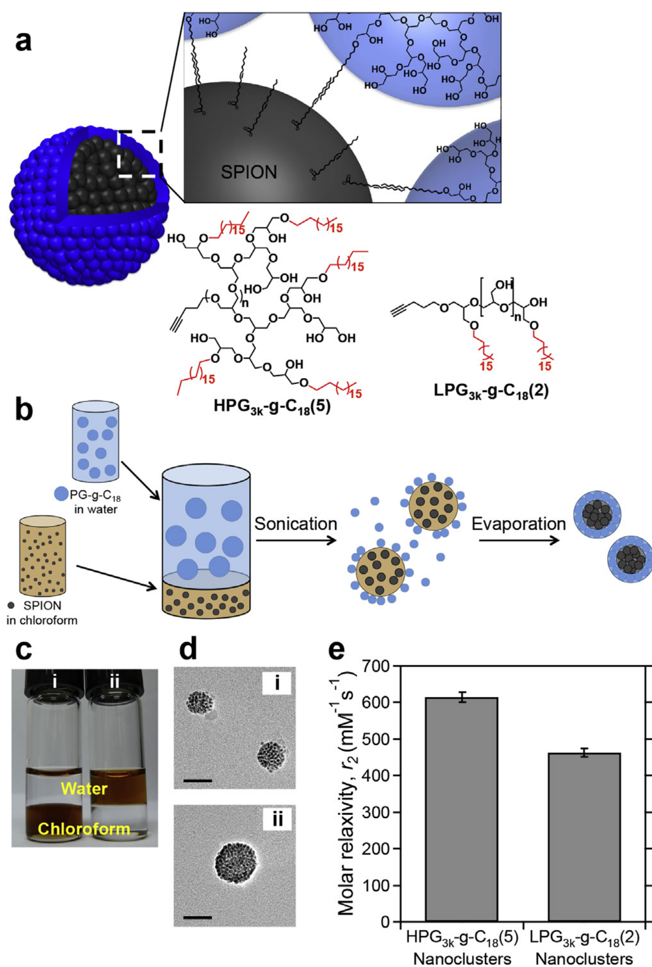

Fig. 1. HPG-coated SPION clusters. (a) Overall scheme of hyperbranched polyglycerol-coated SPION nanoclusters and representative chemical structures of HPG_{3k}-g-C_{18}(5) and LPG_{3k}-g-C_{18}(2). (b) Schematic of the emulsification process to create polyglycerol-coated SPIONs. (c) Oleic acid-capped SPIONs were dispersed in (i) chloroform before emulsification and in (ii) water after emulsification. (d) TEM micrographs of SPIONs coated with (i) HPG_{3k}-g-C_{18}(5) and (ii) LPG_{3k}-g-C_{18}(2). Scale bars represent 50 nm. A minimum of 50 clusters were examined per condition. (e) Effect of molecular architecture on T_2 relaxivity. Error bars represent standard deviation of the fit parameter.

2.2. Synthesis of HPG

The overall synthetic scheme is shown in Fig. S1 and generally follows the methods reported by Kong, Zimmerman, and coworkers [18]. To prepare HPG, sodium hydride (NaH) was mixed with the alkyne initiator, 4-pentyn-1-ol at a 1:10 molar ratio. The mixture was stirred for 15 min followed by addition of doubly distilled glycidol by a syringe pump (1.2 mL/h) while stirring at 70 °C. The molar ratio of glycidol to initiator was varied to achieve different molecular weights. After addition, the reaction continued for 3 h with constant stirring. For each 1 mL of glycidol used, 2 mL of methanol was added and ion exchange Amberlite IR 1200H form prewashed resin was added to the polymer solution and stirred for 1 h at 50 °C. The resin was removed by vacuum filtration and the polymer was fractionally precipitated with cold ether and centrifuged at 4000 rpm for 15 min at 4 °C. The supernatant was decanted and precipitation was repeated 2–3 more times. The resulting HPGs were characterized by mass spectrometry, ^{1}H NMR, and ^{13}C NMR.

2.3. Alkylation of HPG

HPG (40 mg, 0.005 mmol) was dissolved in anhydrous dimethylformamide (DMF, 2.5 mL) to which NaH in 60% mineral oil (7.6 mg, 0.19 mmol) was added. The solution was stirred for 15 min before addition of bromooctadecane (63.3 mg, 0.19 mmol). The average number of conjugated alkyl chains was controlled by varying the ratio of bromooctadecane to HPG. The reaction mixture was then placed in a preheated oil bath at 80 °C for 24 h. The mixture was extracted with hexane 3 times to remove unreacted bromide and the DMF was removed first by rotary evaporator and then by high vacuum. The material was characterized by MALDI-TOF, ^{1}H NMR and ^{13}C NMR.

The degree of substitution of alkyl chain (DS_{C18}) on alkylated HPG was calculated from the integrated peaks of the ^{1}H NMR spectra as follows in Eq. (1):

$$DS_{C18} = \frac{(Peak_{1.3}/30)}{(Peak_{4.0-3.4}/5) - (Peak_{1.3}/30)} \times 100\% \quad (1)$$

2.4. FITC-labeling of alkylated HPG

Fluorescently-labeled HPG was used for analysis of nanocluster transport in the transwell assay described in section 2.14. To modify HPG for this experiment, alkylated HPG was dissolved in anhydrous DMF. NaH (0.17 mmol) was then added and stirred at room temperature for 5–15 min. Fluorescein isothiocyanate (FITC, 0.17 mmol) was added to the flask and protected from light, and the mixture stirred for 24 h at room temperature. The material was purified by dissolving it in methanol, followed by precipitation in cold diethyl ether, and finally, dialysis in a 1000 MWCO membrane against a water/10% methanol/$NaHCO_3$ saturated solution and brine.

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