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Gd^{3+}-1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic-2-hydroxypropyl-β-cyclodextrin/Pluronic Polyrotaxane as a Long Circulating High Relaxivity MRI Contrast Agent

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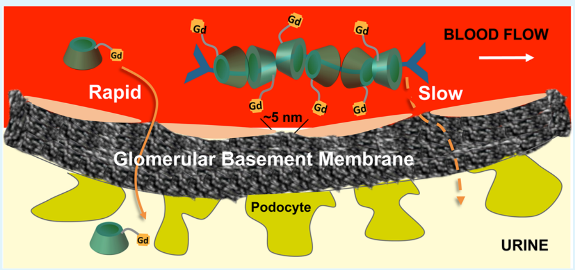
Supporting Information

ABSTRACT: A multivalent magnetic resonance imaging agent based on a 2-hydroxypropyl-β-cyclodextrin (HPCD):Pluronic F127 polyrotaxane carrier has been synthesized, and its blood pool contrast properties have been characterized. This Gd^{3+}-DO3A-HPCD/Pluronic polyrotaxane construct is shown to circulate for more than 30 min and provide >100-fold vascular enhancement relative to the monomeric Gd^{3+}-DO3A-HPCD control that is rapidly cleared via the kidney. The high r_1 relaxivity at 37 °C (23.83 mM^{-1} s^{-1} at 1.5 T; 34.08 mM^{-1} s^{-1} at 0.5 T), extended blood circulation, well-known pharmacology of the polyrotaxane precursors, and absence of acute toxicity make it a highly attractive blood pool contrast agent candidate.



KEYWORDS: *polyrotaxane, 2-hydroxypropyl-β-cyclodextrin, Pluronic, magnetic resonance contrast agent, Gd^{3+}-DO3A, blood pool MR agent, long circulation*

Magnetic resonance imaging (MRI) is a powerful tool for noninvasive, high-resolution three-dimensional (3D) medical imaging of anatomical structures such as organs and tissues within the body. MRI has advantages such as high versatility, high spatial resolution, excellent depth profiling capabilities, and an absence of ionizing radiation as in X-ray and CT imaging.[1−4] MRI has extensive applications in the diagnosis of various neurological, cardiovascular, and oncological diseases.[1] MR contrast agents enhance the image quality by altering the longitudinal (T_1) and transverse (T_2) relaxation times of the protons in nearby water molecules. Contrast agents can be classified as either T_1 agents (e.g., Gd^{3+} chelates) that increase the T_1 relaxation rate and produce positive contrast images or T_2 agents (e.g., superparamagnetic iron oxide nanoparticles) that increase the T_2 relaxation rate and produce negative contrast images. The majority of clinically used MRI contrast agents are Gd^{3+} chelates, which are favored due to their high paramagnetism and excellent relaxation enhancement. Unfortunately, most clinically approved contrast agents suffer from rapid renal clearance and modest contrast enhancement, making them suboptimal for angiographic enhancement and tumor imaging.[5] Thus, the use of long circulating high molecular weight contrast agents are attractive due to their greater clinical flexibility arising from improved pharmacokinetics, potential for tissue selectivity through the use of targeting ligands, and capacity for much higher Gd^{3+} loading to improve contrast and sensitivity.[6] For example, a 75 kDa Gd^{3+}-DTPA-dextran conjugate with 15 Gd^{3+} ions was found to enable vascular imaging in rats for up to 1 h.[7]

Nanomaterial platforms such as dendrimers,[8−10] polymers,[10,11] inorganic particles,[12] and other supramolecular assemblies[13] have been used as carriers of Gd^{3+}; however, many of these carriers suffer from poor renal filtration, hepatobiliary uptake, and *in vivo* accumulation. Their high Gd^{3+} payload can also become a liability if the agent is constructed from nondegradable materials that employ acyclic chelates that are kinetically labile. Contrast agents of this type can leach free Gd^{3+} ions into the bloodstream, where they can provoke nephrogenic systemic fibrosis (NSF) and other cytotoxic responses, as has been observed for low molecular weight acyclic Gd^{3+}-DTPA constructs.[14] Additionally, many of these agents possess a highly flexible structure that tends to reduce their relaxivity. We sought to develop a semiflexible, long circulating contrast agent derived from low toxicity building blocks that could be degraded to rapidly excretable products to obviate these safety and relaxivity problems.

Discher and co-workers have demonstrated that micelles with a flexible rodlike morphology possess longer circulation

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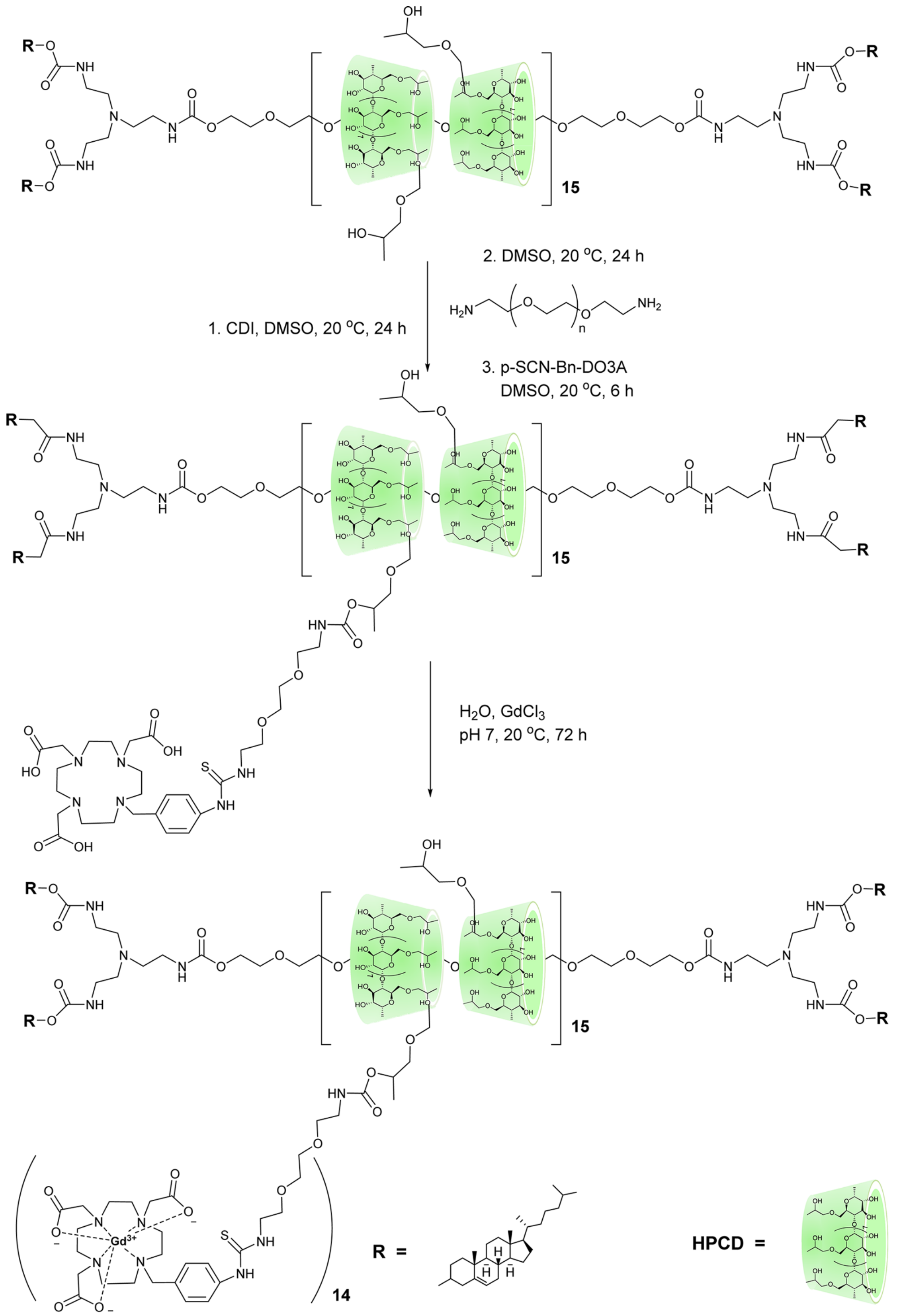

Figure 1. Synthesis of Gd^{3+}-DO3A-HPCD/Pluronic PR.

times than spherical micelles, a property that was attributed to their ability to evade macrophage uptake.[15] More recently, Mitragotri et al.[16] and DeSimone et al.[17] have also demonstrated that nanoparticles with rodlike morphologies were internalized by macrophages and HeLa cells, respectively, at a much lower rate than spherical particles. With these prior findings in mind, we designed a multivalent Gd^{3+} MRI contrast agent based on a flexible rodlike polyrotaxane scaffold. Polyrotaxanes (PR) are noncovalent self-assemblies composed of cyclic molecules threaded onto a polymer "axle" that retains the macrocycles via bulky endcaps positioned at the termini of the "axle".[18,19] Herein, we describe a polyrotaxane-based Gd^{3+}-DO3A MRI imaging agent constructed from 2-hydroxypropyl-*β*-cyclodextrin (HPCD) and Pluronic F127 (Pluronic) (Figure 1), both of which are widely used excipients in FDA-approved formulations for human use. Although Gd^{3+}-DO3A chelate-modified cyclodextrins[20,21] and cyclodextrin polymers[22−25] have been described previously as MRI contrast agents, no polyrotaxanated cyclodextrin contrast agents have been reported. This PR was designed based on the hypothesis that

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its flexible rodlike morphology would confer long circulation properties to the contrast agent. Since HPCD is known to form inclusion complexes with the central poly(propylene oxide) blocks of Pluronic copolymers, this property was utilized to construct a PR bearing carbamate linked endcaps that were attached as described previously,[26,27] except that cholesterol carbonyl chloride was used as endcapping reagent. Analysis by ^{1}H NMR, GPC-MALS/RI, and MALDI-TOF MS indicated that the PR product bore 15 copies of HPCD, with an average molecular weight of 36 kDa and a poly(propylene oxide) block coverage of ∼46%. The polyrotaxane was also analyzed for the presence of free HPCD contamination by RP-HPLC and hydrophilic interaction liquid chromatography; both of these techniques indicated a free HPCD content of <4%. The HPCD/Pluronic PR intermediate was then activated with CDI before modification with an excess of 1,8-diamino-3,6-dioxooctane to increase the water solubility of the material. Finally, this intermediate was coupled with *S*-2-(4-isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane tetraacetic acid (DO3A-Bn-SCN) and then treated with $GdCl_3$ to obtain the Gd^{3+}-DO3A-HPCD/Pluronic PR contrast agent with 14 DO3A attached as determined by ^{1}H NMR and 14 Gd^{3+} ions as determined by ICP-MS. AFM analysis of dried samples on mica indicated that the polyrotaxane prepared had a rodlike morphology with lengths in the range of 30−40 nm and diameters of ∼5 nm, near the threshold dimension that can lead to rapid clearance from the bloodstream due to the effective pore size of the glomerular endothelium.[28]

Relaxivity measurements (Figure 2) of the monomeric and rotaxanated Gd^{3+} agents gave r_1 values of 7.82 and 23.83 mM^{-1} s^{-1} per Gd chelate for Gd^{3+}-DO3A-HPCD and Gd^{3+}-DO3A-HPCD/Pluronic PR, respectively, at 1.5 T, 37 °C (8.46 and 34.08 mM^{-1} s^{-1} at 0.5 T, respectively, Supporting Information). The Gd^{3+}-DO3A-HPCD r_1 relaxivity is in good agreement with the ionic relaxivity reported for other

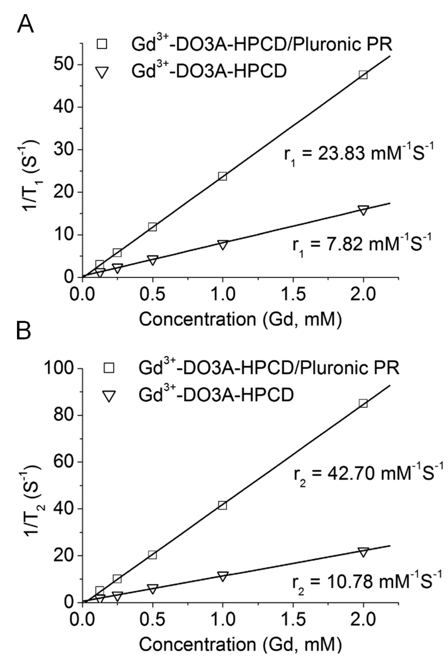

Figure 2. Plots of $1/T_1$ (A) and $1/T_2$ (B) versus the concentration of the contrast agents Gd^{3+}-DO3A-HPCD and Gd^{3+}-DO3A-HPCD/Pluronic PR.

cyclodextrin monomeric contrast agents ([Gd^{3+}-DOTA]$_7$-*β*-CD, r_1 = 12.2 mM^{-1} s^{-1} at 1.5 T;[20] [Gd^{3+}-DTTA]$_7$-*β*-CD, r_1 = 6.2 mM^{-1} s^{-1} at 9.4 T[21]) and a dextran-DTPA derivative with a similar Gd^{3+} content (r_1 = 10.5 mM^{-1} s^{-1} at 0.25 T);[7] a [Gd^{3+}-DOTA]$_8$-sucrose derivative (4.1 mM^{-1} s^{-1}),[29] all measured at 37 °C. The greater than 3-fold improvement in ionic relaxivity of the PR construct relative to the Gd^{3+}-DO3A-HPCD monomer is attributed to the increased polymer rigidity imparted by rotaxanation as well as reduced rotational motion of the threaded, hydrogen-bonded nearest-neighbor cyclodextrin units.

We then evaluated the monomeric and PR Gd^{3+}-DO3A agents in Balb/c mice to determine their contrast enhancement capabilities. MR images (Figure 3) revealed that Gd^{3+}-DO3A-HPCD/Pluronic PR has a substantially longer circulation time in mice than the monomeric Gd^{3+}-DO3A-HPCD derivative. Within 5 min, the monomer had largely cleared from the blood and accumulated in the kidneys and bladder, with little visible intensity in the heart. The polyrotaxane derivative, however, had a substantially better signal enhancement in the blood of the heart at 5 min that only slowly diminished out to the 30 min time point. Improved blood circulation of the Gd^{3+}-DO3A-HPCD/Pluronic PR species enabled the visualization of greater anatomic detail and blood vessel organization. Conversely, the monomeric Gd^{3+}-DO3A-HPCD chelate did not produce significant blood enhancement due to rapid elimination via renal filtration. No acute toxicity was observed in any of the animals tested.

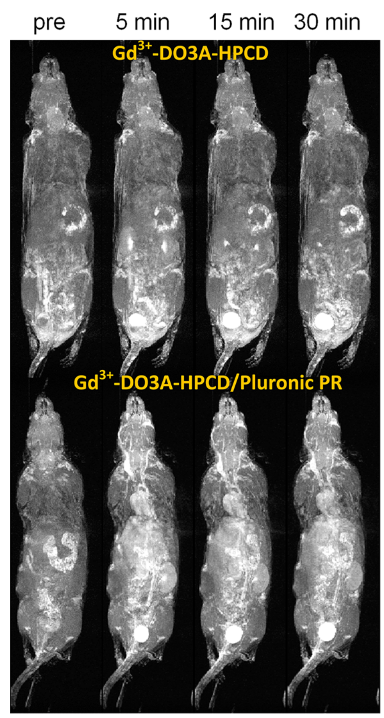

Figure 3. Representative 3D maximum intensity projection images (T_1-weighted) of Balb/c mice injected with Gd^{3+}-DO3A-HPCD (top row) or Gd^{3+}-DO3A-HPCD/Pluronic PR (bottom row) at a 0.03 mM-Gd/kg dose. Images are shown for contrast agent distribution before injection (pre) and at 5, 15, and 30 min after tail vein injection of Gd^{3+} complexes.

Quantitative analysis of the MR images revealed an increased enhancement ratio (ER) in the blood of the heart of approximately 2-fold during the first 30 min for the

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polyrotaxane contrast agent, whereas the ER of the Gd^{3+}-DO3A-HPCD control was 1.25 after 5 min and dropped to background levels within 25 min (Figure 4). Furthermore, the

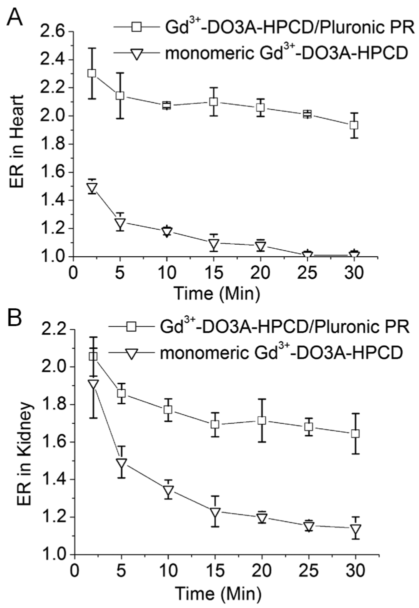

Figure 4. Enhancement ratio (ER = S_{post}/S_{pre}) of the MR signal postinjection to that of preinjection in the blood of the heart (A) and in the kidneys (B) (n = 3). Balb/c mice injected at a 0.03 mM-Gd/kg body weight dose with Gd^{3+}-DO3A-HPCD (monomer, square) or Gd^{3+}-DO3A-HPCD/Pluronic PR (PR, triangle).

ER observed in the kidneys for the Gd^{3+}-DO3A-HPCD/Pluronic PR gradually decreased from 1.9 to 1.65 between 5–30 min, while that observed with the monomeric Gd^{3+}-DO3A-HPCD control dropped rapidly from 1.55 to 1.1 over the same time period, indicating rapid renal filtration of the control. The superior MR contrast enhancement of the Gd^{3+}-DO3A-HPCD/Pluronic PR derivative in the blood at 30 min relative to the control demonstrates the improved pharmacokinetics of the polyrotaxane motif. Given that the polyrotaxanes were observed to undergo slow clearance through the kidneys, we infer from these findings that the Gd^{3+}-DO3A-HPCD/Pluronic PR contrast agent platform possesses the potential for low *in vivo* accumulation, either through direct excretion of intact low molecular weight members of the polyrotaxane population through the glomerular membrane or by enzymatic cleavage of the carbamate bonds linking the endcap to the Pluronic core and subsequent dethreading of the rotaxanated HPCD units[26,27] to produce easily eliminated Gd^{3+}-DO3A-HPCD monomers and the F127 Pluronic precursor. Further experiments are needed to discriminate between these possible mechanisms. These findings also suggest that HPCD/Pluronic PR display long circulation properties that may provide significant advantages in the cyclodextrin-mediated mobilization of aberrantly stored cholesterol, as occurs in tissues affected by Niemann-Pick Type C disorder.[26,27]

■ CONCLUSIONS

We report the development of a long circulating, degradable Gd^{3+}-DO3A-HPCD/Pluronic polyrotaxane that has the intravascular imaging capabilities of a macromolecular contrast agent, while potentially retaining the renal elimination properties of a small molecule agent after endcap cleavage. Taken together, these results suggest that Gd^{3+}-DO3A-HPCD/Pluronic PR may be a promising material for development as a cardiovascular enhancement contrast agent due to its lack of acute toxicity, long circulation properties, and potential for providing a greatly improved safety profile relative to nondegradable polymer contrast agents.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsami.5b05393.

^{1}H NMR, GPC, MALDI-TOF MS, and AFM data of the PR agent (PDF)

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Author Contributions

[§]These authors have contributed equally. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

HP-β-CD, 2-hydroxypropyl-β-cyclodextrin
MALS/RI, multiangle light scattering/refractive index detection
PR, polyrotaxane

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