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Polymer micelles decorated by gadolinium complexes as MRI blood contrast agents: design, synthesis and properties

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New micellar macrocontrast agents with improved contrast at high frequencies were designed by grafting a gadolinium based contrast agent onto functional stealth micelles formed by poly(ethylene oxide)-*b*-poly(ε-caprolactone) (PEO-*b*-PCL) in water. As evidenced by relaxometry measurements and the hemolytic CH50 test, the new contrast agents are of interest as MRI blood pool agents.

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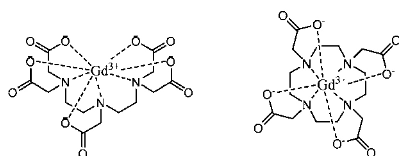
Introduction

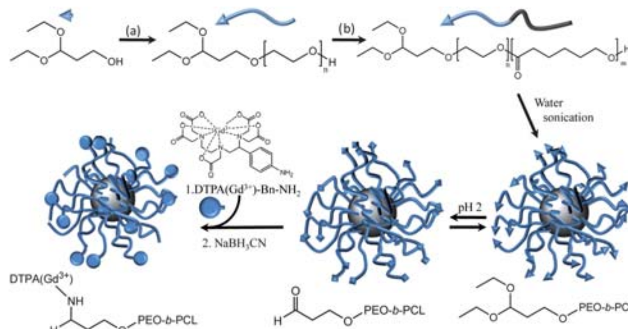
Nuclear Magnetic Resonance Imaging (MRI) is presently the leading imaging modality in diagnostic medicine.[1] MRI is noninvasive and allows imaging biological objects (soft tissues for instance) and processes at the molecular level. The image contrast in MRI depends essentially on differences in relaxation times and proton density between different tissues. To reach the full potential of MRI for advanced imaging applications, the inherently low sensitivity of MRI has to be enhanced with contrast agents (CAs) containing paramagnetic metal ions such as Gd(III).[2]

The contrast agents currently approved for clinical uses are based on low molecular weight Gd(III) chelates, such as Magnevist® and Dotarem® (Scheme 1). However, modern MRI instruments are operating at high magnetic fields[3–5] (*i.e.* frequencies) where contrast agents have a low relaxivity (the longitudinal relaxation rates per second and per mmol of metal ion that governs the contrasting efficiency). Consequently, relatively large amounts of these potentially toxic contrast agents are needed per injection and further improvements are required to alleviate this problem. Three main directions can be considered for that purpose: (1) enhancing the relaxivity of Gd(III) based complexes, (2) improving the circulation lifetime in the case of blood pool agents, and (3) targeting them to the desired tissue. Firstly, the relaxivity of Gd(III) based complexes can be much improved by reducing their tumbling rate in solution. Slowing down rotational motions was successfully achieved by immobilizing gadolinium complexes onto macromolecules of different sizes and shapes (proteins,[6–10] polylysine,[11] dendrimers,[12–14] polysaccharides (*i.e.* dextran[15,16]) and micelles (or liposomes)[4,17–21]). Although those strategies have allowed to push rotational correlation times to their theoretical maximum, they are all too often compromised either by nonoptimal water residency times, poor solubility in water, reduced target affinity and selectivity, or lack of realistic usefulness in the actual application. The pharmacokinetic behavior of these agents is also a concern and has to be determined case-by-case.[21] Secondly, the decoration of high molecular weight metal complexes by stealth molecules (such as polyethylene oxide, PEO) is a way to increase their blood residence time that allows ill tissues to be reached.[22] Finally, the efficacy of the contrast agents can be improved by functionalizing them with targeting peptides[20,23] and/or by grafting them to macromolecules that passively target tumor tissues through a combination of reduced renal clearance and exploitation of the enhanced permeation and retention (EPR) effect, which prevails for fast-growing tumors.[24–26]

In the following studies, we aim at designing novel gadolinium based blood pool contrast agents[3] with improved relaxivity at high frequencies resulting from the grafting in mild conditions of the gadolinium complex (*S*-2-(4-aminobenzyl)-diethylenetriamine



Scheme 1 Clinically used contrast agents for MRI (left: Magnevist® and right: Dotarem®).



Scheme 2 General procedure for the synthesis of the macromolecular contrast agent [(a) (1) Napht-K, EO (THF, 40 °C); and (2) isopropanol; and (b) $Sn(oct)_2$, ε-CL (toluene, 120 °C)].

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pentaacetic acid, p-NH_2-Bn-DTPA(Gd^{3+}), Scheme 2) at the surface of biocompatible micelles formed by an amphiphilic poly(ethylene oxide)-*b*-poly(ε-caprolactone) block copolymer (PEO-*b*-PCL) in water. The relaxivity should be improved by decreasing the tumbling rate of the gadolinium complex while the PEO chains should ensure the repulsion of opsonins allowing for a prolonged blood circulation. These improvements are intensively searched for decreasing the doses of gadolinium required per injection while maintaining satisfactory image acquisition times.

Experimental section

General procedures

Commercial tetrahydrofuran (THF), 3,3-diethoxy-1-propanol, naphthalene, toluene, heptane, isopropanol, tin(II) 2-ethylhexanoate [$Sn(oct)_2$, tin octoate, 95%], 6-aminofluorescein and caprolactone (ε-CL) were purchased from Sigma-Aldrich. *S*-2-(4-Aminobenzyl)-diethylenetriamine pentaacetic acid (p-NH_2-Bn-DTPA) was purchased from Macrocyclics. Ethylene oxide (EO, 99.9%) was purchased from Chemogas and was used as received. ε-Caprolactone (ε-CL) was dried over calcium hydride at room temperature for 48 h and distilled under reduced pressure just before use. THF and toluene were dried with a sodium/benzophenone system and distilled just before use.

Synthesis

Synthesis of α-acetal-PEO-OH. α-$PEO_{(4000)}$-OH was synthesized by ring-opening polymerization of EO as follows. Naphthalene (1.21 g, 9.45 mmol) was dissolved in 250 ml of dried THF in a glass reactor, followed by the addition of 0.3 g of potassium (7.88 mmol). The mixture was stirred at room temperature during 3 hours and the color turned to dark-green corresponding to the formation of the naphthalene/K complex. This mixture was added to a solution of 0.99 ml (6.3 mmol) of 3,3-diethoxy-1-propanol dissolved in 200 ml of dried THF. After 10 min of stirring, the solution was transferred to a 1 l stainless steel reactor and 25 g (0.57 mol) of EO were added to the solution. The polymerization of EO proceeded for 4 h at 50 °C. An excess of isopropanol (10 ml) was then added to stop the reaction and the polymer was quantitatively recovered by precipitation into a large volume of heptane and was dried under vacuum. Polymer was analyzed by ^{1}H NMR and size exclusion chromatography.

^{1}H NMR ($CDCl_3$, TMS, 250 MHz): δ = 1.19 ppm (t, 6H, CH_3–CH_2–), δ = 1.88 ppm (q, 2H, –CH–CH_2–CH_2–), δ = 3.65 ppm (m, 364H, –CH_2–CH_2–O–), δ = 4.63 ppm (t, 1H, –O–CH–CH_2–). $M_{n,NMR}$ = 4000 g mol^{-1}, $DP_{n,NMR}$ = 91, $M_{n,SEC}$ = 3900 g mol^{-1}, M_w/M_n = 1.11.

α-Acetal-PEO_{1600}-OH and α-acetal-PEO_{2500}-OH were synthesized using the same experimental procedure, except that the monomer to initiator ratio was adapted accordingly.

Synthesis of α-acetal-PEO-*b*-PCL. α-Acetal-PEO-*b*-PCL was synthesized by ring-opening polymerization of ε-CL according to the following general procedure described elsewhere.[27,28] 1 g of α-acetal-PEO_{4000}-OH (2.5 × 10^{-4} mol) was dried under reduced pressure at 100 °C for 10 h in a glass reactor and then dissolved in 10 ml of dried toluene. ε-CL (0.65 ml, 0.65 mol) and $Sn(Oct)_2$ (0.83 ml of a 0.06 M solution (in toluene), 5 × 10^{-6} mol (0.02 equiv./OH))[29] were added to the acetal-PEO-OH solution. The polymerization of ε-CL proceeded for 5 h at 120 °C. The copolymer was quantitatively recovered by precipitation into a large volume of heptane and dried over vacuum. The copolymer was analyzed by ^{1}H NMR spectroscopy and SEC.

^{1}H NMR ($CDCl_3$, TMS, 250 MHz): δ = 1.19 (t, 6H, CH_3–CH_2–), δ = 1.37 (m, 46H, –CH_2–CH_2–CH_2–CH_2–CH_2–O–), δ = 1.64 (m, 92H, –CH_2–CH_2–CH_2–CH_2–CH_2–O–), δ = 2.30 (t, 46H, –CH_2–CH_2–CH_2–CH_2–CH_2–O–), δ = 3.65 (m, 364H, –CH_2–CH_2–O–), δ = 4.05 (t, 46H, –CH_2–CH_2–CH_2–CH_2–CH_2–O–), δ = 4.63 (t, 1H, –O–CH–CH_2–). $M_{n,NMR}$ = 6600 g mol^{-1}, $DP_{n,NMR}$ = 114, $M_{n,SEC}$ = 6100 g mol^{-1}, M_w/M_n = 1.16.

Micelle formation. 150 mg of copolymer were dissolved in 3 ml of THF. After complete dissolution, the solution was added dropwise under vigorous sonication in 50 ml of water. The solution was stirred overnight at room temperature to remove THF.

Conversion of acetal into aldehyde end groups. The conversion of the acetal end group into an aldehyde function was conducted after the micelle formation previously described. The pH of the micellar copolymer solution was decreased to 2 by the addition of HCl 0.1 M during 2 h. The pH was then increased to 7 by the slow addition of a NaOH 0.1 M solution. Finally, the solution was dialyzed for 48 h against distilled water (Spectra/Por dialysis membrane, molecular weight cut-off, 6000/8000). The dialysate was exchanged four times. Finally, the solution was lyophilized and analyzed by ^{1}H NMR to quantify the aldehyde conversion.

^{1}H NMR ($CDCl_3$, TMS, 250 MHz): δ = 1.37 (m, 46H), δ = 1.64 (m, 92H), δ = 2.30 (t, 46H), δ = 3.65 (m, 364H), δ = 4.05 (t, 46H), δ = 9.35 (s, 0.6 H). $M_{n,NMR}$ = 6600 g mol^{-1}, $DP_{n,NMR}$ = 114, $M_{n,SEC}$ = 6100 g mol^{-1}, M_w/M_n = 1.16.

Preparation of the Gd^{3+} complex with NH_2-Bn-DTPA. Ligand [*S*-2-(4-aminobenzyl)-diethylenetriamine pentaacetic acid] (NH_2-Bn-DTPA) (644 mg, 1 mmol) was dissolved in 5 ml water. $GdCl_3 \cdot 6H_2O$ (409 mg, 1.1 mmol) was added to this solution and the pH was increased to 6 by the addition of a KOH (1 M) solution. The solution was heated at 40 °C overnight. Finally, the uncomplexed Gd^{3+} ions were removed by eluting the solution through a Chelex 100 (Bio-Rad Laboratories, sodium form) resin. The resulting solution was brought to dryness under reduced pressure and the NH_2-Bn-DTPA(Gd^{3+}) complex was recovered as a pale yellow solid in almost quantitative yield (>95%). MS(ESI$^-$): *m*/*z*: 637 [M − H]$^-$.

Synthesis of macrocontrast agent (DTPA(Gd^{3+})-PEO-*b*-PCL): conjugation of NH_2-Bn-DTPA(Gd^{3+}) to aldehyde-PEO-*b*-PCL. NH_2-Bn-DTPA(Gd^{3+}) was grafted to α-aldehyde-PEO_{4000}-*b*-PCL_{2600} by reductive amination according to the following optimized conditions. 100 mg of α-aldehyde-PEO_{4000}-*b*-PCL_{2600} (15 µmol of aldehyde group) were placed in 2 ml THF and added drop by drop to 40 ml of sodium carbonate/sodium hydrogen carbonate buffer solution (0.1 mol l^{-1}, pH = 7). 9.65 mg of NH_2-Bn-DTPA(Gd^{3+}) (15 µmol) were added to this solution. The solution was stirred for 1 h. Sodium cyanoborohydride (0.15 ml of a 1 M solution in THF, 150 µmol) was added and the mixture

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was allowed to stir for 96 h. Unreacted reagents were removed by dialysis (Spectra/Por, molecular weight cut-off 6000/8000) against water for 48 h. Finally, the micelles were lyophilized. The grafting yield (60%) was determined by quantifying gadolinium in the final purified product by inductive coupling plasma (ICP) analysis.

Characterizations

^{1}H NMR spectra of the different polymers were recorded at 298 K with a Bruker spectrometer (250 MHz) in $CDCl_3$ ((*D*1) 2 s, 16 scans, 5 wt% of polymer). Size exclusion chromatography (SEC) of the polymers was carried out in dimethylformamide containing 25 mM LiBr (flow rate: 1 ml min^{-1}) at 55 °C with a Waters 600 liquid chromatrograph equipped with a 410 refractive index detector and four Waters Styragel columns [HR 1 (100–5000), HR 3 (500–30 000), HR 4 (5000–500 000), and HR 5 (2000–4 000 000) (7.8 × 300 mm)]. Poly(ethylene glycol) standards were used for calibration. Copolymers were analyzed for their size and charge distribution using a Malvern Zetasizer® Nano Series DTS 1060 (Malvern Instruments S.A., Worcestershire, UK). Copolymers concentrations were adjusted to 1 mg ml^{-1} in a solution composed of 750 μl of deionised water and 250 μl of Veronal-buffered saline containing 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+} in order to ensure a convenient scattered intensity on the detector.

Field cycling relaxometry. The water proton NMRD profiles were measured at 25 °C on a Stelar fast field-cycling spectrometer FFC-2000 in water between 0.0002 and 1.88 T (corresponding to 0.01–80 MHz proton Larmor frequencies). The ^{1}H T_1 relaxation times were acquired by the standard inversion recovery method with a typical 90° pulse width of 3.5 μs, using 16 experiments of 4 scans.

Inductive coupling plasma (ICP). The gadolinium concentration of the macrocontrast agent was determined by inductively coupled plasma mass spectrometry (ICP-MS), carried out on a DRC II spectrometer (Perkin Elmer). Samples were prepared by reaction of 1.5 ml of the macrocontrast agent solution with 2 ml of HNO_3 (65%) at room temperature for 2 h. The solution was then diluted to 100 ml with bidistilled water prior to ICP-MS analysis.

Complement consumption testing (CH50 test)

Complement consumption was assessed in normal human serum (NHS) (provided by the Etablissement Français du Sang, CHU, Angers, France) by measuring the residual haemolytic capacity of the complement system after contact with particles. The technique consisted in determining the amount of serum able to lyse 50% of a fixed number of sensitized sheep erythrocytes with rabbit antisheep erythrocyte antibodies (CH50), according to the procedure described elsewhere.[30,31] Complement activation was expressed as a function of the surface in order to compare particles with different mean diameters. Nanoparticle surface areas were calculated as described elsewhere,[32] using the equation: $S = n4\pi r^2$ and $V = n(4/3)(\pi r^3)$ leading to $S = 3m/r\rho$ where S is the surface area (cm^2) and V the volume (cm^3) of n spherical beads of average radius r (cm), weight m (μg) and volumetric mass ρ (μg cm^{-3}).

Results and discussion

Synthesis of the macrocontrast agent (DTPA(Gd^{3+})-PEO-*b*-PCL)

The general procedure for the synthesis of the macrocontrast agent consists in first preparing an aldehyde functionalized poly(ethylene oxide)-*b*-poly(ε-caprolactone) (ald-PEO-*b*-PCL) diblock copolymer, followed by imination with *S*-2-(4-aminobenzyl)-diethylenetriamine pentaacetic acid (NH_2-Bn-DTPA(Gd^{3+})) and reduction with sodium cyanoborohydride (Scheme 2).

The procedure described by Scholz *et al.*[28] for the synthesis of aldehyde-ended poly(ethylene glycol)-*b*-poly(lactide) block copolymer was adapted for the preparation of the aldehyde functionalized poly(ethylene glycol)-*b*-poly(ε-caprolactone) (Scheme 2).

We first synthesized poly(ethylene oxide) of different molecular weights (M_n = 1300–2500–4000 g mol^{-1}) bearing an acetal group at the α position (α-acetal-PEO-OH) by ring-opening polymerization of EO using potassium 3,3-diethoxy-1-propanolate as initiator, as reported elsewhere.[27] Next, the terminal hydroxyl group of α-acetal-PEO-OH was used to initiate the ring-opening polymerization of ε-caprolactone with $Sn(oct)_2$ to produce the corresponding block copolymer α-acetal-PEO-*b*-PCL. The composition and polydispersity of the prepared copolymers have been determined by ^{1}H NMR and SEC, respectively, and are summarized in Table 1 (columns 2 and 3). After precipitation and drying, the copolymer was dissolved in THF and added dropwise to water under vigorous sonication to form the block copolymer micelles with a hydrophobic PCL core and a hydrophilic PEO corona bearing the acetal groups. Deprotection of the acetal groups into aldehyde was performed by decreasing the pH of the solution to 2. Finally, the pH was increased to 7 and the product was dialyzed against water. Lyophilization led to the corresponding aldehyde functional copolymer (α-aldehyde-PEO_{4000}-*b*-PCL_{2600}). ^{1}H NMR analysis

Table 1 Data of acetal-PEO-*b*-PCL, aldehyde-PEO-*b*-PCL and DTPA(Gd^{3+})-PEO-*b*-PCL block copolymers and their micelles

	NMR and SEC data of acetal-PEO-*b*-PCL (in $CDCL_3$ and DMF respectively)		DLS data of aldehyde-PEO-*b*-PCL micelles in water		DLS data of DTPA(Gd^{3+})-PEO-*b*-PCL micelles in water	
Entry	M_n/g mol^{-1} (DP)[a]	M_w/M_n[b]	Diameter[c]/nm	PDI[d]	Diameter[c]/nm	PDI[d]
1	1300-*b*-1500 (30–13)	1.16	15	0.24	20	0.22
2	1300-*b*-3000 (30–26)	1.19	23	0.2	30	0.18
3	2500-*b*-1200 (57–11)	1.17	14	0.21	20	0.28
4	2500-*b*-2000 (57–18)	1.18	26	0.25	35	0.2
5	2500-*b*-5000 (57–44)	1.15	40	0.28	49	0.3
6	4000-*b*-1600 (91–14)	1.16	30	0.3	40	0.27
7	4000-*b*-2600 (91–23)	1.18	37	0.25	50	0.27

[a] Degree of polymerization [DP] determined by NMR. [b] Molecular weight distribution determined by size exclusion chromatography. [c] Micelles diameter. [d] Polydispersity determined by dynamic light scattering.

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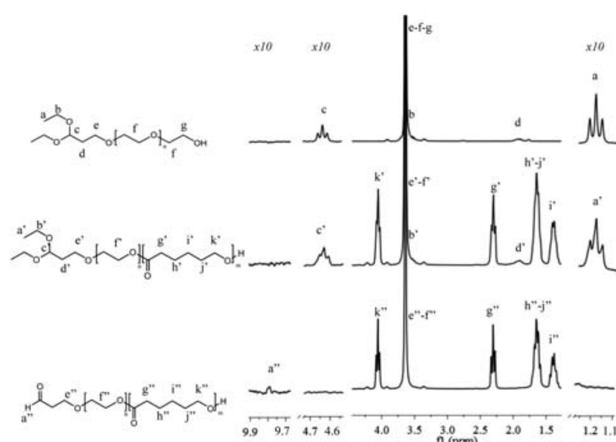

Fig. 1 ^{1}H NMR spectra of α-acetal-PEO$_{4000}$-OH (top), α-acetal-PEO$_{4000}$-*b*-PCL$_{2600}$ (middle) and α-aldehyde-PEO$_{4000}$-*b*-PCL$_{2600}$ (bottom).

(Fig. 1) of the block copolymer before and after deprotection allowed us to demonstrate that the reaction was quantitative thanks to the complete disappearance of the typical peaks of the acetal groups at 1.19 ppm (–O–CH–CH$_2$–C*H*$_3$) and 4.63 ppm (–O–C*H*–CH$_2$–CH$_3$). The appearance of a singlet at 9.35 ppm is characteristic of the aldehyde function that represents however only 60% of the initial acetal groups. This discrepancy is the result of the aldehyde/hydrate equilibrium that establishes itself easily in water.[33]

Size exclusion chromatography analysis of the block copolymer before (B curve, Fig. 2) and after (C curve, Fig. 2) deprotection clearly shows that no degradation of the PCL block occurred in these mild experimental conditions.

The aldehyde functional PEO-*b*-PCL micelles in water (0.5 mg ml^{-1}) with a hydrophobic PCL core and a hydrophilic PEO corona bearing the aldehyde groups have been characterized by dynamic light scattering measurements (Table 1, columns 4 and 5). Depending on the size and composition of the block

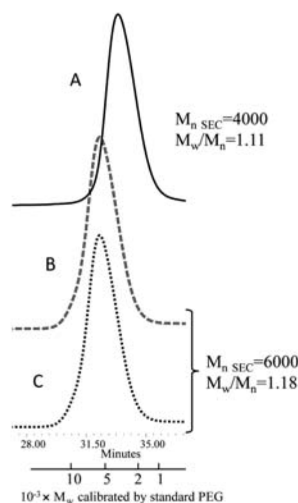

Fig. 2 Gel permeation chromatograms in DMF of acetal-PEO$_{4000}$-OH (A, line), acetal-PEO$_{4000}$-*b*-PCL$_{2600}$ (B, double lines) and aldehyde-PEO$_{4000}$-*b*-PCL$_{2600}$ (C, dashed line).

copolymers, micelles have a diameter between 14 and 40 nm with a size distribution between 0.2 and 0.3. As expected, for a same PEO block, increasing the size of the hydrophobic block increases the size of the micelles.

To these micellar solutions was added an equimolar (relative to the aldehyde functions) aqueous solution of the contrast agent (NH$_2$-Bn-DTPA(Gd^{3+})) prepared by chelation of gadolinium cations by the commercially available NH$_2$-Bn-DTPA. After reaction of the amino group of NH$_2$-Bn-DTPA(Gd^{3+}) with the aldehyde moieties at the micelle surface, an excess of sodium cyanoborohydride (NaBH$_3$CN) was added to reduce the so-formed imine into the corresponding stable amine moieties. For each copolymer, the reaction yield was equal to 60% as determined by ICP analysis of the gadolinium content after removing the ungrafted NH$_2$-Bn-DTPA(Gd^{3+}) by micelles dialysis. The average diameter and size distribution of the DTPA(Gd^{3+})-PEO-*b*-PCL were measured by DLS (Table 1) and compared with the average diameter of the aldehyde-copolymers. Micelles have a diameter between 20 and 50 nm with a size distribution between 0.18 and 0.3. As expected, grafting of NH$_2$-Bn-DTPA(Gd^{3+}) on aldehyde-PEO-*b*-PCL increases the size of the micelles.

Relaxivities of the DTPA(Gd^{3+})-PEO-*b*-PCL micelles

The contrasting efficiencies of the synthesized macrocontrast agents (DTPA(Gd^{3+})-PEO-*b*-PCL) in micellar form are compared in Table 2. The relaxivity (r_1) of the different contrast agents was calculated from formula 1 after determining the longitudinal relaxation time (T_1) of water protons.[34,35]

$$\frac{1}{T_{1,\mathrm{obs}}} = r_1\left[\mathrm{Gd}^{3+}\right] + \frac{1}{T_{1,\mathrm{H_2O}}} \quad (1)$$

where $T_{1,\mathrm{obs}}$ and $T_{1,\mathrm{H_2O}}$ are the longitudinal relaxation times in the presence and in the absence of the MR contrast agent [2.86 s], respectively, and [Gd^{3+}] is the concentration of the contrast agent in mM.

The relaxivities of the different macrocontrast agents at 20 MHz were found to vary between 9 and 12 mmol^{-1} s^{-1} and are significantly higher than that of the free and low molecular weight contrast agent DTPA(Gd^{3+}) (r_1 = 4.3 mmol^{-1} s^{-1}) (Table 2). The relaxivity enhancements noted for the macrocontrast agents result from an increase of their rotational correlation lifetime due to their bulkiness. The observed enhancements are only slightly lower than other micellar systems based on gadolinium (r_1 ≈ 18 mmol^{-1} s^{-1} at 20 MHz) because of the high flexibility of the PEO chains of our PEO-*b*-PCL micelles.

Table 2 Data of DTPA(Gd^{3+})-PEO-*b*-PCL block copolymer micelles and their relaxivities at 20 MHz

Entry	Copolymer PEO-*b*-PCL	Relaxation time/ms (20 MHz, 25 °C)	Relaxivity/mM^{-1} s^{-1}
1	1300-*b*-1500	84.3	11.9 (±0.2)
2	1300-*b*-3000	107.2	9.3 (±0.2)
3	2500-*b*-1200	85.1	11.8 (±0.1)
4	2500-*b*-2000	84.5	11.8 (±0.2)
5	2500-*b*-5000	106	9.4 (±0.1)
6	4000-*b*-1600	97.2	10.3 (±0.2)
7	4000-*b*-2600	99.2	10.1 (±0.1)

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Comparing the characteristics of the micelles prepared using the different PEO-*b*-PCL block copolymers (Table 1) and the relaxivities of the corresponding DTPA(Gd^{3+})-PEO-*b*-PCL micelles at 20 MHz (Table 2) clearly shows that there is no simple relationship between the size of the micelles and the relaxivities. Most of the relaxivities are in the same range ($10 \leq r_1 \leq 12$ mmol^{-1} s^{-1}), except for the micelles formed from the block copolymers with the PCL sequence longer than the PEO one ($r_1 \approx 9.5$ mmol^{-1} s^{-1}, Table 2 entries 2 and 5).

Full relaxometric data were measured for two DTPA(Gd^{3+})-PEO-*b*-PCL based micelles over a large magnetic field range (from 0.01 to 100 MHz) and were compared to DTPA(Gd^{3+}) (Fig. 3). At low frequency (0.01 to 5 MHz), the relaxivity of the macrocontrast agent is about twice higher than that of free DTPA(Gd^{3+}). Importantly, the effect of the immobilization of gadolinium on micelles has an even more pronounced effect on relaxivity at high frequencies (10 to 80 MHz). Indeed, the micelle relaxivities remain strongly enhanced when compared to DTPA(Gd^{3+}) whose relaxivity drops drastically to 4 mM^{-1} s^{-1}. The maximum relaxivity for the best macrocontrast agent we have developed, *i.e.* DTPA(Gd^{3+})-PEO_{1300}-*b*-PCL_{1500}, was obtained at 40 MHz ($r_1 = 12.6$ mM^{-1} s^{-1}) with a 300% relaxivity increase upon attachment of DTPA(Gd^{3+}) onto the micelles by comparison with free DTPA(Gd^{3+}). Once again, when the PCL sequence of the PEO-*b*-PCL block copolymer is longer than the PEO one, the relaxivities are lower. Symmetrical PEO-*b*-PCL or block copolymers with a shorter PCL sequence are thus preferred for optimized relaxivities.

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Complement activation test[36]

The improvement of the relaxivity of a contrast agent by immobilizing it onto polymeric micelles is the first step towards the reduction of the dose needed for satisfactory image acquisition. The second step consists in preventing the recognition of the macrocontrast agent by the immune system that is responsible for its rapid elimination from the blood circulation, restricting timing for studies. For that purpose, poly(ethylene oxide) is the most popular polymer used for imparting stealthiness to molecules or particles injected in the body.[37–41]

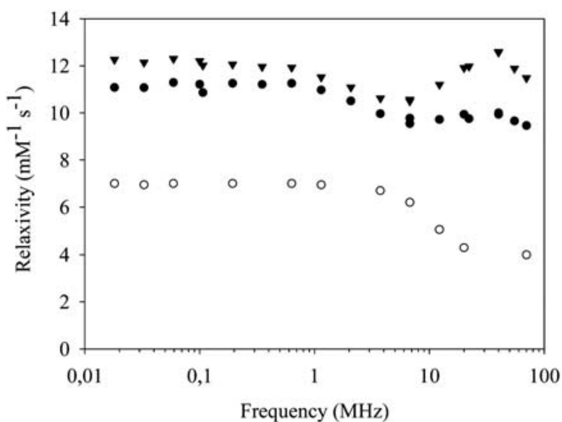

Fig. 3 Comparison of the ^{1}H NMRD profiles of DTPA(Gd^{3+})PEO_{1300}-*b*-PCL_{1500} (full triangles), DTPA(Gd^{3+})PEO_{2500}-*b*-PCL_{5000} (full circles) and NH_2-Bn-DTPA(Gd^{3+}) (empty circles) at 298 K.

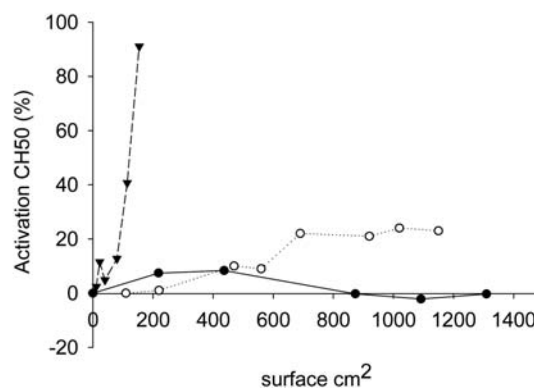

Fig. 4 Consumption of CH50 unit's *vs.* surface area of DTPA(Gd^{3+})-PEO_{2500}-*b*-PCL_{5000} (full circles), P(MMA-*co*-MA) (full triangles) and PEO_{4900}-*b*-PCL_{3900} (empty circles).

The PEO corona at the macrocontrast agent surface should therefore improve its blood circulation time. One of the macrocontrast agents (DTPA(Gd^{3+})-PEO_{2500}-*b*-PCL_{5000}) was therefore evaluated by the hemolytic CH50 test and compared to a PEO_{4900}-*b*-PCL_{3900} block copolymer known for stealthiness.[42] Both copolymers are very poor activators of the complement system in comparison with a P(MMA-*co*-MA) copolymer chosen as positive control[43] (Fig. 4). Interestingly, Fig. 4 clearly shows that the grafting of the gadolinium complex onto PEO-*b*-PCL micelles does not activate the complement. This very low activation means that the macrocontrast agent is expected to have a long blood circulation time and could be ready to be evaluated by *in vivo* test like plasma clearance test.

Conclusion

Aldehyde functionalized micelles based on a hydrophilic corona of poly(ethylene oxide) and a hydrophobic core of poly(ε-caprolactone) were prepared in water and grafted with a gadolinium based contrast agent. Relaxometry measurements of these novel micellar macrocontrast agents have evidenced the strong increase (up to 300%) of relaxivity at high frequencies compared to low molecular weight contrast agents due to the decrease of their tumbling rate in solution. Moreover, the hemolytic CH50 test has demonstrated that the PEO chains at the surface of the macrocontrast agents prevent their recognition by the immune system, imparting long-circulating properties to molecules. The improved relaxivity and the potential long circulation time of the macrocontrast agent make them good potential candidates for MRI blood pool contrast agent.

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