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Amphiphilic Hyperbranched Fluoropolymers as Nanoscopic ^{19}F -Magnetic Resonance Imaging Agent Assemblies

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Abstract

Three hyperbranched fluoropolymers were synthesized and their micelles were constructed as potential ^{19}F MRI agents. A hyperbranched star-like core was first synthesized *via* ATR-SCVCP of 4-chloromethyl styrene (CMS), lauryl acrylate (LA) and 1,1,1-tris(4'-(2"-bromoisobutyryloxy) phenyl)ethane (TBBPE). The polymerization gave a small core with M_n of 5.5 kDa with PDI of 1.6, which served as a macroinitiator. Trifluoroethyl methacrylate (TFEMA) and *tert*-butyl acrylate (*t*BA) in different ratio were then "grafted" from the core to give three polymers with M_n of *ca.* 120 kDa and PDI values of *ca.* 1.6–1.8. After acidolysis of the *tert*-butyl ester groups, amphiphilic, hyperbranched star-like polymers with M_n of *ca.* 100 kDa were obtained. These structures were subjected to micelle formation in aqueous solution to give micelles having TEM-measured diameters ranging from 3–8 nm and DLS-measured hydrodynamic diameters from 20–30 nm. These micelles gave a narrow, single resonance by ^{19}F NMR spectroscopy, with a half width of approximately 130 Hz. The T_1/T_2 parameters were *ca.* 500 ms and 50 ms, respectively, and were not significantly affected by the composition and sizes of the micelles. ^{19}F MRI phantom images of these fluorinated micelles were acquired, which demonstrated that these fluorinated micelles may be useful as novel ^{19}F MRI agents for a variety of biomedical studies.

Introduction

Magnetic resonance imaging (MRI) based on ^1H signals has been shown to be a powerful tool for non-invasive, non-destructive diagnostic applications in various clinical and biomedical research settings.¹ Paramagnetic contrast agents that employ Gd^{III} to accelerate proton relaxation often are employed to reveal physiological and pathological structural details that may be nonobvious in routine scans, as a consequence of similarities in T_1 relaxation times in adjacent tissues.^{2,3}

In addition to Gd^{III} -assisted ^1H -MRI techniques, contrast agents such as those based on ^{19}F -NMR signals are emerging as attractive alternatives, due to the high sensitivity of the ^{19}F chemical shift to local microenvironment, near-zero background signal, high gyromagnetic ratio, good biocompatibility, and high natural abundance.^{4,5} ^{19}F -MRI agents are the focus of

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Supporting Information Available: NMR spectra of polymers and DLS plots of prepared micelles. This material is available free of charge via the Internet at <http://pubs.acs.org>.

extensive research intended for a range of applications. For example, ^{19}F -MRI has been demonstrated in several studies that include real time monitoring of drug delivery,^{1,6} imaging of vascular injury, tumor oxygenation studies,^{7,8} lung imaging,⁹ stem cell labeling and tracking,^{10,11} among others. As ^{19}F imaging agents, a variety of fluorinated small molecule compounds has been proposed, typically constructed from perfluorocarbons (PFCs) with high fluorine content and a single fluorine resonance, such as perfluoro-15-crown-5-ether (PFCE) or perfluorobenzene (PFB).^{12–14} Highly fluorinated compounds are advantageous in these applications, since the high fluorine concentration enhances the signal to noise ratio (SNR) and decreases the scanning time necessary for high resolution imaging.¹⁵ PFCs with multiple resonances, such as perfluoropolyether (PFPE), perfluorooctyl bromide (PFOB), and trifluoromethylsulfonate (TFMS), have also been reported as ^{19}F imaging agents.^{16–20} These agents require selective excitation, due to the different fluorine chemical shifts present in the molecule, which may impair the SNR.²⁰ Currently, PFCE or PFB are the most commonly used PFCs in ^{19}F -MRI research.^{10,12–14,21} These PFC molecules have poor solubility in water and, as such, are typically emulsified into lipid micro/nanoparticle formulations for biological applications.^{5,7,21} Such lipid constructs can give one single, narrow peak with a half width less than 150 Hz, which is sufficiently narrow for MRI studies. However, such lipid based formulations sometimes have drawbacks, such as long T_1 relaxation times (more than 1000 ms at 1.5 T) that necessitate the use of Gd^{III} to shorten the T_1 relaxation, and large sizes (*ca.* 200–300 nm) that limit imaging capabilities to the vasculature with *intravenous* administration.

We are especially interested in developing novel fluorinated nanomaterials to serve as MRI agents, for which the size and architecture of the nanomaterial can be tuned for different biomedical applications. Such nanomaterials are also designed for loading with therapeutic agents and functionalization with targeting ligands for tissue-selective delivery and imaging purposes. Polymeric micelles, self assembled from amphiphilic copolymers with high fluorine content and high solubility in water, are examples of such nanomaterials that may serve as an alternative to lipid based formulations, because of their greater design possibilities, increased storage stabilities, and smaller sizes. Hyperbranched polymers and their well-defined analogs, dendrimers, are promising materials for such micellar assemblies since they often form unimolecular micelles that are not limited by the critical micelle concentration (CMC), a characteristic that is advantageous in *in vivo* applications.^{22,23}

We selected atom transfer radical self-condensing vinyl (co)polymerization (ATR-SCVCP) as the method for preparing such hyperbranched fluoropolymers (HBFP), since this simple and elegant technique allows for the large scale preparation of hyperbranched polymers from commercial vinyl monomers in one pot by the aid of an inimer.^{24–30} In this work, ATR-SCVCP was used to first construct a core to act as a hydrophobic pocket for passive therapeutic loading and to serve as a platform for further construction of a shell consisting of a copolymer of a fluorinated monomer to introduce the ^{19}F labels and hydrophilic acrylic acid for increased water solubility and micellar stability. The corresponding polymeric micelles, assembled from these amphiphilic star-like hyperbranched fluorinated polymers,^{31–35} were then investigated in terms of their physicochemical characteristics, for their ^{19}F -NMR properties such as T_1/T_2 relaxation properties, and also tested by ^{19}F -MRI phantom imaging to evaluate the quality of the images arising from these nanostructures. High signal-to-noise ratio (SNR) images were obtained from these nanoscale polymeric micelles, which provide promise for their development as a novel type of ^{19}F -MRI agent.

Experimental

Materials and methods

All chemicals were purchased from Sigma–Aldrich Chemical Company, unless otherwise noted. 1,1,1-tris(4'-(2"-bromoisobutyryloxy)phenyl)ethane (TBBPE) [**1**] was synthesized according to the literature method.³⁶

IR spectra were obtained on a Perkin-Elmer Spectrum BX Fourier-transform infrared (FTIR) spectrometer using NaCl plates, with the sample being deposited from CH₂Cl₂ and allowing for evaporation of the solvent.

¹H NMR spectra were recorded at 300 or 500 MHz on a Varian Unity-plus 300 or Varian Inova 500 spectrometer, respectively, with the solvent proton signal as standard. ¹³C NMR spectra were recorded at 125 MHz on a Varian Inova 500 spectrometer, with the solvent carbon signal as standard. ¹⁹F NMR spectra were recorded at 470 MHz on a Varian Inova 500 spectrometer with CF₃COOH as an external standard.

Gel permeation chromatography (GPC) was conducted on a Waters 1515 HPLC (Waters Chromatography, Inc.) equipped with a Waters 2414 differential refractometer, a PD2020 dual-angle (15° and 90°) light scattering detector (Precision Detectors, Inc.), and a three-column series PL gel 5μm Mixed C, 500 Å, and 10⁴ Å, 300×7.5 mm columns (Polymer Laboratories, Inc.). The system was equilibrated at 35 °C in stabilized THF, which served as the polymer solvent and eluent with a flow rate of 1.0 mL min⁻¹. Polymer solutions were prepared at a known concentration (*ca.* 5 mg mL⁻¹) and an injection volume of 200 μL was used. Data collection and analyses were performed, respectively, with Precision Acquire software and Discovery 32 software (Precision Detectors, Inc.). Interdetector delay volume and the light scattering detector calibration constant were determined by calibration using a nearly monodisperse polystyrene standard (Pressure Chemical Co., M_p = 90 kDa, M_w/M_n < 1.04). The differential refractometer was calibrated with standard polystyrene reference material (SRM 706 NIST), of known specific refractive index increment *dn/dc* (0.184 mL g⁻¹).

Differential scanning calorimetric (DSC) studies were performed on a Mettler Toledo DSC822 (Mettler Toledo, GmbH.) calibrated according to standard procedures. The heating and cooling rates were 10 °C min⁻¹ with a temperature range of -50 °C to 200 °C. The glass transition temperature (*T_g*) was taken as the midpoint of the inflection tangent on the third heating run.

Dynamic light scattering (DLS) measurements were acquired using a Brookhaven Instruments (Worcestershire, U.K.) system, including a model BI-200SM goniometer, a model BI-9000AT digital correlator, a model EMI-9865 photomultiplier, and a model 95-2 Ar ion laser (Lexel, Corp.; Farmindale, NY) operated at 514.5 nm. Measurements were made at 25 ± 1 °C. Prior to analysis, solutions were filtered through a 0.22 μm Gelman Nylon Acrodisc® 13 membrane filter to remove dust particles. Scattered light was collected at a fixed angle of 90°. The digital correlator was operated with 522 ratio spaced channels, and initial delay of 5 μs, a final delay of 100 ms, and a duration of 10 minutes. A photomultiplier aperture of 400 μm was used, and the incident laser intensity was adjusted to obtain a photon counting of between, 200 and 300 kcps. The calculations of the particle size distributions and distribution averages were performed with the ISDA software package (Brookhaven Instruments Co.), which employed single-exponential fitting, cumulants analysis, non-negatively constrained least-squares (NNLS) and CONTIN particle size distribution analysis routines. The data is presented as the average values from four measurements.

Transmission electron microscopy (TEM) measurements were conducted on a Hitachi H600 microscope. Micrographs were collected at 100,000× magnification and calibrated using a 41

nm polyacrylamide bead from NIST. Carbon-coated copper grids were treated with oxygen plasma prior to deposition of the micellar samples. The samples were stained with 1% phosphotungstic acid (PTA) for 1 minute, and then the solution was wicked away and the samples were allowed to dry under ambient conditions. The number-average particle diameters (D_{av}) and standard deviations were generated from the analysis of a minimum of 100 particles from at least three different micrographs.

Atomic force microscopy was performed under ambient conditions in air. The AFM instrumentation consisted of a Nanoscope III BioScope system (Digital Instruments, Veeco Metrology Group; Santa Barbara, CA) and standard silicon tips (type, OTESPA-70; L, 160 μ m; normal spring constant, 50 N/m; resonance frequency, 246–282 kHz). The sample solutions were spin cast from native concentration onto freshly-cleaved mica and allowed to dry under ambient conditions.

Elemental analyses were conducted by Galbraith Laboratories (Knoxville, TN) as a commercial service.

The ^{19}F relaxation parameters, T_1/T_2 measurements of the micelles were conducted on a Varian Inova 500 MHz spectrometer using inversion recovery spectroscopy with 9 inversion times for T_1 , and multi-echo spin echo spectroscopy with 10 different echo times for T_2 . For T_1 measurements, $sw = 11981.4$, Hz, $pw = 12.5$ μ s, $nt = 16$. For T_2 measurements, $sw = 11981.4$ Hz, $pw = 8.0$ μ s, $nt = 16$.

MRI images were acquired at 11.7 T on a Varian Inova system. Micelle solutions were loaded into a 20×10 mm plastic tubing and the images were acquired with the use of a 3-cm custom-designed birdcage coil. Proton spin-echo images were first acquired with imaging parameters: $TR = 150$ ms; $TE = 30$ ms; $FOV = 4 \times 4$ cm; data acquisition matrix = 512×256 ; slice thickness = 1 mm, yielding an in-plane resolution of 156×156 μ m. ^{19}F MRI images were acquired with the use of a 1.5-cm single-turn solenoid coil, dual-tuned to ^1H and ^{19}F . $TR = 1500$ ms; $TE = 16$ ms; $FOV = 2 \times 2$ cm; data acquisition matrix = 32×32 ; slice thickness = 10 mm, yielding an in-plane resolution = 62.5×62.5 μ m, $nt = 1024$.

Synthesis

Synthesis of the hyperbranched polymer [2]—A flame-dried Schlenk flask equipped with a magnetic stir bar was charged with 4-chloromethyl styrene (CMS, 1.54 g, 10 mmol), lauryl acrylate (2.40 g, 10 mmol), 1,1,1-tris(4'-(2"-bromoisobutyryloxy)phenyl)ethane (TBBPE) [1] (376 mg, 1 mmol), bipyridine (bipy, 343 mg, 2.2 mmol), and 1,4-dioxane (10 mL). After one freeze-pump-thaw cycle, CuCl (99 mg, 1.0 mmol), and CuCl_2 (13.4 mg, 0.1 mmol) were added to the solution and the reaction mixture was subjected to three more cycles of freeze-pump-thaw. The reaction was stirred for 10 min to ensure homogeneous mixing and was then placed in an oil bath set at 65 $^\circ\text{C}$. The progress of the polymerization was monitored by removing small aliquots by a nitrogen-washed gas tight syringe and analyzed by GPC and ^1H NMR spectroscopy. After 6 h, the polymerization was quenched by placing the flask into liquid nitrogen and exposing the reaction mixture to air. The copper catalyst was removed by filtration through a short plug of basic alumina. The solvent was removed under reduced pressure and the polymer was purified by repeated precipitations ($\times 3$) into 800 mL of hexane. The polymer was finally collected by centrifugation (3000 rpm $\times$ 5 min) to give **2** as a white powder after drying under reduced pressure (1.4 g, 60%). Conversion of the monomers: CMS 75%, LA 35 %. M_n^{GPC} : 5.5 kDa, M_w^{GPC} : 8.8 kDa, PDI: 1.6. M_n^{NMR} : 5.6 kDa. T_g : 20 $^\circ\text{C}$. ^1H -NMR (500 MHz, CD_2Cl_2): δ 0.9 (br, s, CH_3), 1.0–2.5 (br, m, CH_2 and CH of backbone), 3.8–4.2 (s, br, CH_2OCO), 4.4–4.6 (m, PhCH_2), 6.4–7.5 (m, br, ArH) ppm. ^{13}C -NMR (125 MHz, CD_2Cl_2): δ 14.6, 23.3, 26.5, 29.9, 30.3, 31.1, 39.7–43.3, 46.2, 46.7, 60.1, 60.9, 62.2, 64.8–65.2, 73.2, 121.4, 126.7–130.5, 136.1–149.7, 175.7, 175.9 ppm. IR (NaCl plate, CH_2Cl_2): 678,

826, 1016, 1104, 1169, 1208, 1266, 1447, 1503, 1729, 1729, 2853, 2925 cm^{-1} . Elem. Anal. Calcd. for $\text{C}_{323}\text{H}_{428}\text{Br}_3\text{Cl}_{19}\text{O}_{22}$ ($M_n = 5.5$ kDa): C, 69.57; H, 7.74; Cl, 12.08; found: C, 70.05; H, 7.74, Cl, 12.95.

Synthesis of hyperbranched fluoropolymer [3a] general polymerization

procedure—A flame-dried Schlenk flask equipped with a magnetic stir bar was charged with polymer **2** (106 mg, 0.28 mmol Br/Cl), trifluoroethyl methacrylate (TFEMA, 3.40 g, 20 mmol), *tert*-butyl acrylate (*t*BA, 2.60 g, 20 mmol), bipy (96 mg, 0.62 mmol), and toluene (6 mL). After one cycle of freeze-pump-thaw, CuCl (28 mg, 0.28 mmol) and CuCl₂ (3.70 mg, 0.028 mmol) was added to the reaction, and the reaction mixture was subjected to three more cycles of freeze-pump-thaw. The reaction was stirred for 10 min to ensure homogeneous mixing and was then placed in an oil bath set at 65 °C. The progress of the polymerization was monitored by removing small aliquots by a nitrogen-washed gas tight syringe and analyzed by GPC and ¹H NMR spectroscopy. After 24 h, the polymerization was quenched by placing the flask into liquid nitrogen and exposing the reaction mixture to air. The copper catalyst was removed by filtration through a short plug of basic alumina. The solvent was removed under reduced pressure and the polymer was purified by repeated precipitations into 800 mL of hexane. The polymer was collected by centrifugation (3000 rpm × 5 min) to give **3a** as white sticky solid after drying under reduced pressure (1.79 g, 50%). Conversion of the monomers: TFEMA 60%, *t*BA 56%. M_n^{GPC} : 122 kDa, M_w^{GPC} : 195 kDa PDI: 1.6. M_n^{NMR} : 120 kDa. T_g : 48 °C. ¹H-NMR (500 MHz, CD₂Cl₂): δ 0.8–2.4 (br, m, CH₂ and CH of backbone), 4.3–4.7 (s, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH) ppm. ¹³C-NMR (125 MHz, CD₂Cl₂) δ 14.6, 18.4, 20.2, 20.9, 26.4–30.3, 36.2, 37.7, 38.4, 40.4, 40.9, 41.4, 41.6, 42.6, 43.5, 45.9, 46.5, 60.8, 61.1, 68.2, 80.7, 123.8 (q, $t = 275$ Hz), 127.5–129.9, 174.2, 174.5, 175.1 ppm. ¹⁹F-NMR (470 MHz, CD₂Cl₂), δ 6.0 (s) ppm. IR (NaCl plate, CH₂Cl₂): 750, 845, 974, 1148, 1257, 1257, 1276, 1367, 1392, 1456, 1482, 1728, 2953, 2978 cm^{-1} . Elem. Anal. Calcd. for $\text{C}_{5302}\text{H}_{7613}\text{Br}_3\text{Cl}_{19}\text{F}_{1233}\text{O}_{1562}$ (M_n : 120 kDa): C, 52.77; H, 6.36; F, 19.41; found: C, 52.14, H, 6.34, F, 19.19.

Synthesis of hyperbranched fluoropolymer [3b]—2 (106 mg, 0.28 mmol Br/Cl), TFEMA (1.68 g, 10 mmol), *t*BA (3.60 g, 28 mmol), bipy (96 mg, 0.62 mmol), CuCl (28 mg, 0.28 mmol), CuCl₂ (3.7 mg, 0.028 mmol), and toluene (6 mL) were reacted according to the general polymerization procedure outlined in **3a** to give polymer **3b** as a sticky white solid after drying under reduced pressure (1.5 g, 53%). Conversion of the monomers: TFEMA 61%, *t*BA 50 %. M_n^{GPC} : 127 kDa, M_w^{GPC} : 203 kDa, PDI: 1.6. M_n^{NMR} : 122 kDa. T_g : 52 °C. ¹H-NMR (500 MHz, CD₂Cl₂): δ 0.8–2.4 (br, m, CH₂ and CH of backbone), 4.3–4.8 (s, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH) ppm. ¹³C-NMR (125 MHz, CD₂Cl₂): δ 14.3, 18.4, 20.2, 20.9, 26.4–30.3, 36.2, 37.7, 38.4, 40.4, 40.9, 41.4, 41.6, 42.6, 43.5, 45.9, 46.5, 60.8, 61.1, 68.2, 80.7, 123.8 (q, $t = 275$ Hz), 127.8–129.8, 173.8, 174.0, 174.2, 174.5, 175.1 ppm. IR (NaCl plate, CH₂Cl₂): 757, 848, 974, 990, 1146, 1257, 1267, 1392, 1455, 1728, 2978 cm^{-1} . ¹⁹F-NMR (470 MHz, CD₂Cl₂) δ 6.0 (s) ppm. Elem. Anal. Calcd. for $\text{C}_{6253}\text{H}_{9838}\text{Br}_3\text{Cl}_{19}\text{F}_{690}\text{O}_{1782}$ ($M_n = 127$ kDa): C, 58.88; H, 7.77; F, 10.28; found: C, 58.43; H, 7.74, F, 10.27.

Synthesis of hyperbranched fluoropolymer [3c]—2 (106 mg, 0.28 mmol Br/Cl), TFEMA (0.94 g, 5.6 mmol), *t*BA (4.30 g, 33.6 mmol), bipy (96 mg, 0.62 mmol), CuCl (28 mg, 0.28 mmol) CuCl₂ (3.7 mg, 0.028 mmol) and toluene (6 mL) were reacted according to the general polymerization procedure outlined in **3a** to give polymer **3c** as a sticky white solid after drying under reduced pressure (1.7 g, 56%). Conversion of the monomers: TFEMA 63%, *t*BA 54 %. M_n^{GPC} : 144 kDa, M_w^{GPC} : 260 kDa, PDI: 1.8. M_n^{NMR} : 140 kDa. T_g : 56 °C. ¹H-NMR (500 MHz, CD₂Cl₂): δ 0.8–2.4 (br, m, CH₂ and CH of backbone), 4.0–4.7 (m, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH) ppm. ¹³C-NMR (125 MHz, CD₂Cl₂): δ 14.5, 18.4, 20.2, 20.9, 26.4–30.3, 36.2, 37.7, 38.4, 40.4, 40.9, 41.4, 41.6, 42.6, 43.5, 45.9, 46.5, 60.8, 61.1, 68.2, 80.7, 123.8 (q, $t = 275$ Hz), 127.8–130.0, 173.8, 174.2, 174.5, 175.1 ppm. ¹⁹F-NMR (470 MHz,

CD₂Cl₂): δ 6.0 (s) ppm. IR (NaCl plate, CH₂Cl₂): 755, 780, 944, 1148, 1165, 1250, 1259, 1278, 1399, 1466, 1489, 1726, 2955, 2974 cm⁻¹. Elem. Anal. Calcd. for C₇₃₇₃H₁₁₉₈₈Br₃Cl₁₉F₄₈₀O₂₀₈₂ (M_n: 140 kDa): C, 61.50; H, 8.39; F, 6.33; found: C, 61.10; H, 8.43, F, 6.31.

General procedure for removal of the tert-butyl groups, synthesis of amphiphilic fluoropolymer [4a]—A flame-dried round bottom flask equipped with a magnetic stir bar was charged with polymer **3a** (336 mg, 0.0028 mmol) and dichloromethane (3.4 mL). To the stirred solution, trifluoroacetic acid (TFA, 1.71 g, 15 mmol) was added. The reactions were stirred at room temperature for 3 h. Solvent and TFA were removed under reduced pressure and the polymer was purified by precipitation in hexanes (3 × 100 mL). The polymer was then collected by centrifugation (3000 rpm × 5 min) to give **4a** as a white powder after drying under reduced pressure (190 mg, 68%). *T_g*: 110 °C. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 0.8–2.3 (br, m, CH₂ and CH of backbone), 4.3–4.7 (s, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH), 12.0–12.5 (s, br, COOH) ppm. ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 17.0–18.4, 27.1–32.0, 44.2, 44.9, 46.2, 50.1, 51.1, 52.9, 59.3–62.3, 123.5 (q, J = 275 Hz), 128.0–130.4, 135.3, 174.5–176.5 ppm. ¹⁹F-NMR (470 MHz, DMSO-*d*₆): δ 6.0 (s) ppm. IR (NaCl plate, CH₂Cl₂): 665, 744, 745, 936, 1123, 1254, 1272, 1456, 1555, 1655, 1704, 2896, 2980, 3123 cm⁻¹. Elem. Anal. Calcd. for C₃₈₆₃H₄₇₃₈Br₃Cl₁₉F₁₂₃₀O₁₅₆₂ (M_n: 100 kDa): C, 46.19; H, 4.75; F, 23.26; found: C, 47.14, H, 4.68, F, 22.71.

Synthesis of amphiphilic fluoropolymer [4b]—3b (220 mg, 0.0018 mmol), dichloromethane (5.4 mL) and TFA (4.1 g, 36 mmol) were reacted according to the general procedure outlined in **4a** to give **4b** as a white powder after drying under reduced pressure (140 mg, 81 %). *T_g*: 127 °C. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 0.8–2.3 (br, m, CH₂ and CH of backbone), 4.3–4.7 (m, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH), 12.0–12.5 (s, br, COOH) ppm. ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 17.0–18.4, 27.1–32.0, 44.2, 44.9, 46.2, 50.1, 51.1, 52.9, 59.3–62.3, 123.4 (q, J = 275 Hz), 128.1–129.9, 135.3, 174.5–176.5 ppm. ¹⁹F-NMR (470 MHz, DMSO-*d*₆): δ 6.0 (s) ppm. IR (NaCl plate, CH₂Cl₂): 669, 744, 745, 1129, 1256, 1273, 1555, 1655, 1724, 2980, 3125 cm⁻¹. Elem. Anal. Calcd. for C₃₆₅₃H₄₆₃₈Br₃C₁₁₉F₆₉₀O₁₇₈₂ (M_n: 91 kDa): C, 48.17; H, 5.13; F, 14.39; found: C, 47.86; H, 5.25; F, 13.89.

Synthesis of amphiphilic fluoropolymer [4c]—3c (100 mg, 0.00071 mmol), dichloromethane (2.7 mL) and TFA (2.1 g, 18 mmol) were reacted according to the general procedure outlined in **4a** to give **4c** as a white powder after drying under reduced pressure (60 mg, 85%). *T_g*: 131 °C. ¹H-NMR (500 MHz, DMSO-*d*₆): δ 0.8–2.3 (br, m, CH₂ and CH of backbone), 4.2–4.7 (m, br, CH₂CF₃, PhCH₂), 6.4–7.5 (m, br, ArH), 12.1–12.5 (s, br, COOH) ppm. ¹³C-NMR (125 MHz, DMSO-*d*₆): δ 17.0–18.4, 27.1–32.0, 44.2, 44.9, 46.2, 50.1, 51.1, 52.9, 59.3–62.3, 123.4 (q, J = 275 Hz), 128.4–130.3, 135.3, 174.5–176.5 ppm. ¹⁹F-NMR (470 MHz, DMSO-*d*₆): δ 6.0 (s) ppm. IR (NaCl plate, CH₂Cl₂): 677, 680, 744, 755, 936, 1127, 1259, 1282, 1459, 1557, 1701, 1745, 2898, 2980, 3129 cm⁻¹. Elem. Anal. Calcd. for C₃₈₃₃H₄₉₄₈Br₃C₁₁₉F₄₈₀O₂₀₄₂ (M_n: 94 kDa): C, 49.12; H, 5.32; F, 9.73; found: C, 48.74; H, 5.68; F, 9.63.

Preparation of micelle [5a], General procedure for micelle formation—A round bottom flask equipped with a magnetic stir bar was charged with polymer **4a** (20 mg) and DMF (20 mL). An equal volume of nanopure water (20 mL) was then added dropwise to the stirred solution *via* a syringe pump over the course of 2.5 h. After stirring the micelle for 24 h the solution was transferred to a dialysis bag (MWCO 30 kDa) and dialyzed against nanopure water for 72 h to remove DMF, and afford the micelle solution **5a** with a final polymer concentration of 0.31 mg mL⁻¹. TEM: D_{av}: 8 ± 2 nm, DLS: D_{h(n)}: 16 ± 2 nm; D_{h(v)}: 19 ± 2 nm; D_{h(i)}: 281 ± 35 nm.

Preparation of micelle [5b]—Micelles from polymer **4b** was prepared according to the general procedure outlined in **5a** to afford micelle solution **5b** with a final polymer concentration of 0.35 mg mL⁻¹. TEM: D_{av} : 6 ± 1 nm, DLS $D_{h(v)}$: 20 ± 3 nm. DLS: $D_{h(n)}$: 15 ± 3 nm; $D_{h(v)}$: 20 ± 3 nm; $D_{h(i)}$: 190 ± 29 nm.

Preparation of micelle [5c]—Micelles from polymer **4c** was prepared according to the general procedure outlined in **5a** to afford micelle solution **5c** with a final polymer concentration of 0.33 mg mL⁻¹. TEM: D_{av} : 3 ± 1 nm, DLS: $D_{h(n)}$: 21 ± 3 nm; $D_{h(v)}$: 25 ± 1 nm; $D_{h(i)}$: 180 ± 41 nm.

Results and Discussion

Synthesis of the hyperbranched fluoropolymers

Self-condensing vinyl (co)polymerization typically employs an initiating monomer (inimer) in conjunction with a vinyl monomer to build up a highly branched structure by controlled radical or free radical polymerization.^{24–26,28–30} Previously, we have successfully prepared HBFP *via* ATR-SCVCP of pentafluorostyrene (PFS) and a fluorinated inimer.^{24–26,30} These polymers had rather broad polydispersity indices, in the range of 2.2–2.6, and non-uniform GPC profiles. In an effort to prepare HBFP with better control of the shape of the distribution and lower PDI's, we chose to utilize a multifunctional initiator in conjugation with the (co) monomers and inimer.³² This idea stems from the work on hyperbranched polyesters^{37,38} and other hyperbranched systems^{39–41} that are prepared by melt condensation in bulk, in which it has been shown that the addition of a multifunctional core to the condensation polymerization can control the initial stages of the polymerization effectively and result in much more uniform materials. For this application of ATR-SCVCP, a trifunctional initiator, 1,1,1-tris(4'-(2"-bromoisobutyryloxy)phenyl)ethane (TBBPE), was used (Scheme 1).

TBBPE has been shown to be an efficient initiator for ATRP of various vinyl monomers including styrenes and acrylates.^{36,42} By using this initiator (10% molar stoichiometry relative to the monomer) combined with a catalyst system of bipyridine/CuCl/CuCl₂ for the mixed halogen ATR-SCVCP of 4-chloromethyl styrene (CMS), and lauryl acrylate (LA), it was possible to reduce the PDI from 2.2 to 1.6 and obtain polymers with uniform size exclusion chromatography profiles (Figure 1). This polymerization gave the hyperbranched polymer (HBP) **2** with a M_n of 5.5 kDa and a PDI of 1.6.

This HBP was engineered to form a hydrophobic core domain, from which the fluorinated and hydrophilic components could extend after a subsequent copolymerization with polymer **2** as macroinitiator, and a deprotection step. The construction of a hyperbranched core-shell morphology with the fluorinated polymer in the outer shell or sub-shell domain of the HBP is based on the hypothesis that in order to achieve a narrow ¹⁹F-NMR resonance and good MRI images, the fluorinated component should not be located in the poorly-solvated core of a polymeric micelle but instead should be located in the shell or sub-shell domain to improve their solvation and mobility of the fluorinated groups. The hydrophobic core domain may also be utilized to solvate poorly soluble therapeutic components for passive drug delivery. Polymer **2** was used as macroinitiator for the copolymerization of trifluoroethyl methacrylate (TFEMA) and *tert*-butyl acrylate (*t*BA). TFEMA was selected as a monomer because of its single, narrow ¹⁹F-signal resulting in improved SNR, whereas *t*BA (after acidolysis) can serve as a hydrophilic segment stabilizing the polymeric micelle in solution. The polymerizations were conducted by using the same catalyst system as for the preparation of the macroinitiator. Due to the difference in reactivity ratios of methacrylates and acrylates, the conversion of TFEMA was found to be faster than *t*BA, suggesting that these monomers should form a tapered copolymer, having greater incorporation of the TFEMA units early and finishing with *t*BA units extending as the terminal chain segments. The polymerization from **2** was conducted with

three different feed ratios of TFEMA/*t*BA in order to vary the fluorine content, to yield polymers **3a–3c**, with molecular weights of 120–140 kDa and PDI's in the range of 1.6–1.8 (Figure 1, Table 1). The DP numbers listed in Table 1 are the average DP's as determined by NMR, and depending on the molecular weight and number of endgroups of the initiating core there can be considerable difference in the PDI of each block extension. The *tert*-butyl groups of these polymers were subsequently removed by acidolysis in TFA/CH₂Cl₂, to yield the amphiphilic HBFPs **4a–4c**, having similar molecular weights, and engineered with a hydrophobic core, a fluorinated component in the sub-shell domain exhibiting a single ¹⁹F-resonance, and a hydrophilic poly(acrylic acid) outer shell with tailored fluorine concentration.

Preparation and characterization of the polymeric micelles

Polymers **4a–4c** were self assembled into polymer micelles by the slow addition of an equal volume of water to a solution of each polymer in DMF (1 mg mL⁻¹). The shapes and sizes of these micellar assemblies were evaluated by transmission electron microscopy (TEM), atomic force microscopy (AFM) and dynamic light scattering (DLS), and the results are summarized in Table 2. Figure 2 depicts the TEM images obtained for micelles **5a–5c** and, as seen in the figure, the micelles were small (3–8 nm) with circular features. As expected, the micelle (**5c**) derived from the polymer with the lowest fluorine content was found to be the smallest, since the negatively-stained TEM images mainly contrast the hydrophobic segments of the polymeric micelle in its dry state. The dynamic light scattering of these micelles revealed that the size in solution is much larger (*ca.* 20–25 nm). The large hydrodynamic diameters, relative to the TEM diameters, is a consequence of the swollen nature of the PAA-rich segments in water. The micelle with the highest ratio of PAA/fluorine is, therefore, also the largest in size (Supporting information, Table 1). The intensity average hydrodynamic volume of the micelles shows that there are also minor populations of larger polymer assemblies present in the samples, which could not be seen in the number- and volume-based hydrodynamic volume histograms. Figure 3 depicts the AFM images for micelles **5a–5c**, showing collapsed globular micellar assemblies with a width of approximately 30 nm. Quantitative AFM imaging was difficult to perform, due to the flattened nature of the micelles (height ~ 0.8 nm in each case), and the aggregation effect on mica seen for sample **5c**.

¹⁹F-NMR and ¹⁹F-MRI imaging

Previously,²⁴ we have prepared fluorinated micelles based on amphiphilic ethylene oxide-derivatized hyperbranched tetrafluorostyrene-based materials (TFS), which failed to produce adequate MRI images. These micelles exhibited broad peaks in their ¹⁹F-NMR spectra (*ca.* 1000 Hz in half width), possibly due to the poorly solvated fluorine groups that form a hydrophobic core upon self assembly. Furthermore, these TFS-based polymeric nanoparticles exhibited two sets of ¹⁹F resonances from the two fluoroaromatic species, thus, decreasing the effective fluorine concentration and, thereby, reducing the SNR.

In contrast, the micelles derived from polymers **4a–4c** exhibited narrow width in their ¹⁹F-NMR spectra, typically in the range of 120–150 Hz (Figure 4), suggesting that the fluorinated groups were well solvated with a degree of mobility. Since the polymers were prepared from the same macroinitiator and polymerized to similar molecular weights, one would expect that the relaxation behavior of the corresponding micelles would be similar. Indeed, that is the case; the T₁ and T₂ values of the three micelles were in the same range, suggesting all fluorine groups are experiencing the same physical and chemical environments (Table 2). The T₁ values were about 500 ms, much shorter than that of perfluoro-15-crown-5-ether or other perfluorinated small molecules,¹⁸ allowing improved imaging contrast and shorter imaging times. The T₂ values were also approximately the same for micelles **5a–5c**, suggesting that these fluorines had the same degrees of mobility. This result was expected, because the fluorine groups would be located in similar sub-shell domains within the hyperbranched polymer framework,

independent of the feed ratio of *t*BA and TFEMA. Compared to PFCE, the T_2 for these micelles was only slightly shorter (64 ms).⁴³ PFCE is a small molecule and upon emulsifying it into lipid nanoparticles, each PFCE molecule retains conformational degrees of freedom and can rotate relatively freely. The micelles in this study are derived from a polymeric structure, in which the fluorine groups are covalently linked to a polymeric backbone through a small spacer that restricts its mobility. The highly branched framework should, however, contribute to more mobility, compared to a polymeric micelle constructed from a linear polymer.

These micelles were then further evaluated with respect to their ^{19}F imaging capability. The ^{19}F -MRI phantom imaging studies were conducted in a 1 cm diameter plastic tube in an 11.7-Tesla Varian Inova MR system and all micelles exhibited excellent ^{19}F -MR imaging ability with SNR in the range of 32–11 (Figure 5, Table 2). Moreover, these polymeric micelles were not used in conjunction with contrast enhancers such as Gd^{III} . As seen in the figure, micelles with increasing fluorine concentration (**5a**) show better SNR than micelles with lower fluorine content (**5c**), and one can derive a linear relationship between the fluorine concentration and SNR (Figure 6). However, compared to PFCF emulsions, wherein the fluorine content can be 50 % or higher, these micelles have much lower fluorine concentrations. The limited fluorine incorporation necessitated long scanning times (1024 scans, 13 h) to acquire the images shown in Figure 5, although images with lower SNR were obtainable at significantly shorter scan times (*ca.* 3 h with SNR = 16 for micelle **5a**).

Conclusions

Hyperbranched-star amphiphilic fluoropolymers with core-shell morphology have been prepared in two steps *via* ATR-SCVCP. As a first step, 4-chloromethyl styrene (CMS), and lauryl acrylate (LA) were polymerized to form a hyperbranched macroinitiator having a molecular weight of 5.5. kDa with good control over PDI by the addition of a trifunctional coinitiator. From this hyperbranched macroinitiator, trifluoroethyl methacrylate (TFEMA) and *tert*-butylacrylate (*t*BA) in different feed ratios were chain extended to form 120–140 kDa amphiphilic hyperbranched-star block polymers with high fluorine content. These hyperbranched polymers were then self assembled into polymer micelles of *ca.* 20 nm hydrodynamic diameters, which were demonstrated to have one single narrow ^{19}F -NMR signal and good T_1/T_2 relaxation times. Moreover, these nanomaterials were further demonstrated as novel imaging agents for ^{19}F -MRI with good SNR ratios. Unfortunately, multi-hour-long scan times were required, due to the limited ^{19}F concentrations. With such long scan times, these materials are not suitable as blood pool imaging agents. When conjugated with targeting moieties, such as antibodies or peptides for tissue-selective targeting, resulting in much higher local fluorine concentrations, however, they may still be suitable for imaging purposes. With these encouraging results, we are currently optimizing these materials with respect to their fluorine concentration, while also exploring these micellar assemblies for drug delivery applications, for which we have obtained promising initial results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
GPC curves of polymers **2**, **3a–3c**.

Figure 2.
TEM images of micelles **5a–5c** (left-right).

Figure 3.
AFM images of micelles **5a–5c** (left to right).

Figure 4.
 ^{19}F -NMR spectra of the micelles **5a–5c**.

Figure 5.
 ^{19}F -MRI phantom images of micelles **5a–5c** (left to right).

Figure 6.
Signal to noise ratio (SNR) vs. fluorine concentration.

Scheme 1.

Synthetic procedure for preparing the hyperbranched amphiphilic fluoropolymers, illustrating one of many possible isomers (for clarification, oligo/polymerization of repeat units is omitted; please see supporting information for more detailed structural information), and their aqueous solution assembly into nanoparticles. In the schematic illustration of **5a**, **5b** and **5c**, the dark red core units are meant to represent the hydrophobic lauryl acrylate and *p*-chloromethylstyrene based components, with the green and blue chains representing the trifluoroethyl methacrylate and acrylic acid copolymer arms extending from the hyperbranched core.

Table 1

Summary of the polymers prepared.

Sample	M_n NMR [kDa]	M_n GPC (RI) [kDa]	M_w GPC (RI) [kDa]	PDI	DP_n TFEMA NMR	DP_n BA/AA NMR	T_g [°C]
2	5.6	5.5	8.8	1.6	-	-	20
3a	120	122	195	1.6	410	360	48
3b	122	127	203	1.6	230	650	52
3c	144	144	260	1.8	160	850	56
4a	100	^a	-	^a	410	360 ^b	110
4b	91	^a	-	^a	230	650 ^b	127
4c	94	^a	-	^a	160	850 ^b	131

^a Unsuitable for GPC with THF as the eluent;

^b DP_n of acrylic acid.

Table 2

Summary of the micelles prepared.

Sample	D _n TEM [nm]	(D _h) _n DLS [nm]	(D _h) _h DLS [nm]	(D _h) _l DLS [nm]	NMR half width [Hz]	T ₁ [ms]	T ₂ [ms]	SNR
5a	8 ± 2	16 ± 2	19 ± 2	281 ± 35	140	542 ± 0.2	50 ± 0.6	32
5b	6 ± 1	15 ± 3	20 ± 3	190 ± 29	130	521 ± 1.1	53 ± 0.5	25
5c	3 ± 1	21 ± 3	25 ± 1	180 ± 41	120	583 ± 1.3	56 ± 0.7	11