Perfluorocarbon-Loaded Shell Crosslinked Knedel-Like Nanoparticles: Lessons Regarding Polymer Mobility and Self-Assembly

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ABSTRACT: Reversible addition-fragmentation chain transfer polymerization was employed to synthesize a set of copolymers of styrene (PS) and 2,3,4,5,6-pentafluorostyrene (PPFS), as well as block copolymers with tert-butyl acrylate (PrBA)-b-PS-co-PPFS, with control over molecular weight and polydispersity. It was found that the copolymerization of styrene and PFS allowed for the preparation of gradient copolymers with opposite levels of monomer consumption, depending on the feed ratio. Conversion to amphiphilic block copolymers, PAA-b-(PS-co-PPFS), by removing the protecting groups was followed by fitting with monomethoxy poly(ethylene glycol) chains. Solution-state assembly and intramicellar crosslinking afforded shell crosslinked knedel-like (SCK) block copolymer nanoparticles. These fluorinated nanoparticles (ca. 20 nm diameters) were studied as potential magnetic resonance imaging (MRI) contrast agents based on the 19F-nuclei; however, it was found that packaging of the hydrophobic fluorinated polymers into the core domain restricted the mobility of the chains and prohibited 19F NMR spectroscopy when the particles were dispersed in water without an organic cosolvent. Packing of perfluoro-15-crown-5-ether (PFCE) into the polymer micelle was demonstrated with good uptake efficiency; however, it was necessary to swell the core with a good solvent (DMSO) to increase the mobility and observe the 19F NMR signal of the PFCE. © 2009 Wiley Periodicals, Inc. J Polym Sci Part A: Polym Chem 47: 1023–1037, 2009

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INTRODUCTION

Nanoparticle-based in vivo diagnostic imaging is one part of the broad research field of nanomedicine, which is currently the focus of numerous interdisciplinary research groups worldwide.¹–⁶ Nanoscale diagnostic constructs are being developed for a range of imaging modalities such as positron emission tomography (PET), single photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI), among others.¹,⁴,⁵,⁷–¹⁰ Apart from the size, the advantages of using nanoparticles for such applications include their surface multifunctionality and
internal capacity, which provide the possibility to redirect the biodistribution of the particle by attaching tissue-specific ligands to the surface of the particle, and also to pack therapeutic cargos within the confined volume of the nanoparticle.2,3,6,11–15

Although MRI can suffer from sensitivity issues, MRI-based diagnostics have the benefit of both not relying on ionizing radiation and displaying larger contrast between different types of biological soft-tissues, which is important in cancer diagnostics and imaging of the brain, muscle, and cardiovasculature.4 Clinical MRI is typically based on 1H nuclei and is sometimes also used in conjunction with paramagnetic contrast agents such as GdIII (which increases contrast in T1-weighted imaging, where T1 is the spin-lattice relaxation time) or superparamagnetic iron oxide nanoparticles (which provide contrast in T2-weighted imaging, where T2 is the spin–spin relaxation time).16–20 Other contrast agents that are under development are based on other nuclei, for instance 19F, which is thought to be the most useful in functional MRI applications and for applications involving in vivo drug monitoring.1,4,21–23 Fluorinated contrast agents have the advantage of having little or no background signal in tissue, a high gyromagnetic ratio, good biocompatibility, and a NMR-shift that is highly sensitive to changes in local microenvironment.24,25 As 19F contrast agents, fluorinated compounds, such as small molecule perfluorocarbons (PFCs) with high fluorine content and a single fluorine resonance, have been utilized. Examples of such small molecules are perfluoro-15-crown-5-ether (PFCE)26–28 and perfluorobenzene (PFB),29 among others.27,29,30 For MRI applications, a high fluorine concentration is advantageous as it enhances the signal-to-noise ratio (SNR) and decreases the scanning time necessary for high resolution image acquisition. For in vivo applications, these PFCs have been previously emulsified into lipid particle formulations with sizes in the range of several hundred nanometers.27,31,32 The large size of these lipids restricts these contrast agents from imaging smaller features than the vasculature with intravasational administration. In comparison, systems based on polymer- and protein-based fluorinated materials suffer from low elemental concentration of fluorine, because of dilution by other atoms, limiting the fluorine concentration attainable and the applicability as 19F MRI contrast agents.33–39 These polymers (self-assembled into polymer micelles or as free polymers) are, therefore, less suitable as blood pool contrast agents, and must either be concentrated by cellular uptake (for cell tracking in vivo) or fitted with targeting ligands for tissue-specific uptake and local concentration enrichment.22,35,40–42

Another unique possibility to create small nanoscale (<30 nm) MRI contrast agents is to sequester highly fluorinated small molecules within the core domain of a polymeric nanoparticle.30 Shell crosslinked knedel-like (SCK) nanoparticles are one such construct, in which hydrophobic therapeutics can be loaded within the core domain.43–46 The sequestering of small molecules within the nanoparticle framework may be achieved by either selective swelling of the core with organic solvent or by performing the self-assembly in the presence of the PFC, after which the establishment of hydrophobic interactions between the core and the small molecule entities stabilizes the host-guest system.44 SCK nanoparticles are derived from polymeric micelles assembled in aqueous solution from amphiphilic block copolymers; these micelles are then crosslinked throughout the shell domain.47,48 The introduction of covalent crosslinks within the nanostructures provides stabilization of the structure, allowing for higher loading capacity, and also circumventing limitations that the critical micelle concentration (CMC) imposes on polymeric micelle assemblies for in vivo applications.45,49 SCKs can be prepared from a range of amphiphilic block copolymers that are easily accessible by controlled radical polymerization techniques.43,45,46,50,51 This versatility allows for the preparation of a wide range of nanostructures, for which the hydrophilic/hydrophobic block ratio, degree of polymerization, and functionality of the core and shell domains can each be tuned.53,45,50,51 In addition, these robust structures can be fitted with multiple detection elements and/or targeting moieties for in vivo diagnostic applications.9,10,25,52–55

In this work, we were interested in creating SCK nanoparticles for which the core domains are optimized to passively harbor a high loading of PFCs. Such nanoparticle constructs may then further serve as 19F MRI contrast agents for targeted in vivo applications, after the attachment of tissue-specific targeting groups, such as peptides and/or antibodies, to enhance local concentration. We hypothesized that the loading capacity of these nanoparticles for PFCs would be enhanced if the core domain consisted of amorphous

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fluorinated hydrophobic polymer. Therefore, the core domain of these nanoparticles was constructed as a block copolymer of PFS and styrene. As coincidence, it was found that the unique reactivity ratios of these monomers gave tapered/gradient block copolymers, for which the corresponding nanoparticle construct would either have the fluorinated polymer segments topologically placed closer to the core-shell interface or buried within the core domain, allowing for further insight into how to optimize nanoparticles for cargo carrying applications and the effects on $^{19}$F NMR properties.

**EXPERIMENTAL**

**Instrumental**

$^1$H NMR, $^{13}$C NMR, and $^{19}$F NMR spectra were recorded as solutions in CDCl$_3$, CD$_2$Cl$_2$, CD$_3$Cl$_2$, TFA-$d_4$, or DMSO-$d_6$ on a Varian Unity-plus 300 MHz spectrometer, with the solvent proton signal as internal standard.

Gel permeation chromatography (GPC) was conducted on a Waters 1515 HPLC (Waters Chromatography) equipped with a Waters 2414 differential refractometer and a three-column series PLgel 5, 10, and 300 Å, 300 × 7.5 mm columns (Polymer Laboratories). The system was equilibrated at 35 °C in 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. 1–3 mg mL$^{-1}$) and an injection volume of 200 µL was used. Data collection and analysis were performed, respectively, with Precision Acquiresoftware and Discovery 32 software (Precision Detectors).

The height measurements ($H_{hv}$) and distributions for the SCKs were determined by tapping-mode atomic force microscopy (AFM) 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 µm; normal spring constant, 50 N m$^{-1}$; resonance frequency, 246–282 kHz). The sample solutions were either spin-cast from native concentration or drop (50–100 µL) deposited onto freshly cleaved mica and allowed to settle freely for 30 s, after which the excess solution was removed by a filter paper and the mica surface was allowed to dry in air.

Hydrodynamic diameters ($D_h$) and distributions for the SCKs in aqueous solutions were determined by dynamic light scattering (DLS). The DLS instrumentation consisted of a Brookhaven Instruments Limited (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. Before analysis, solutions were filtered through a 0.45-µm nylon filter and centrifuged in a model 5414 microfuge (Brinkman Instruments; Westbury, NY) for 4 min 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 0.5 µs, a final delay of 800 ms, and a duration of 10 min. A photomultiplier aperture of 400 µm was used, and the incident laser intensity was adjusted to obtain a photon counting of 300 kcps. Only measurements in which the measured and calculated baselines of the intensity autocorrelation function agreed to within 0.1% were used to calculate particle size. Particle size distributions were performed with the ISDA software package (Brookhaven Instruments Company), which employed CONTIN particle size distribution analysis routine. The data presented are the average of three or four runs, and the standard deviation is the variation between these runs and not the error in each.

The $^{19}$F relaxation parameters experiments, such as the $T_1/T_2$ measurements, of the micelles were conducted on a Varian Inova 500 MHz spectrometer using inversion recovery spectroscopy with 10 inversion times for $T_1$, and multiecho spin echo spectroscopy with 10 different echo times for $T_2$. For $T_1$ measurements, $sw = 20,000$ Hz, $pw = 13.75$ µs, $nt = 32$. For $T_2$ measurements, $sw = 20,000$ Hz, $pw = 13.75$ µs, $nt = 32$.

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 −20 to 150 °C. The glass transition temperatures ($T_g$) were determined as the midpoint of the tangent to the inflection, during the second heating run.

**Materials**

Polymerizations were performed on a double manifold (0.1 mmHg vacuum, 99.99% N$_2$), with glassware and syringes that were dried in an
oven (100 °C) for at least 1 h, and with syringes that were washed with N₂ (3 ×), before use. Styrene (Sty, 99%), pentfluorostyrene (PFS, 99%), and tert-butyl acrylate (tBA, 99%) were received from Sigma-Aldrich Company (St. Louis, MO) and distilled from calcium hydride, and stored under N₂ before use. Trifluoroacetic acid (TFA; 95%; distilled from calcium hydride, and stored under vacuum from Sigma-Aldrich Company (St. Louis, MO) and 1,1,2-trichloro-1,1,2-trifluoroethane): δ Ar-ortho (-74.4 (−75.4), ArF-para (−87.4 (−92.0), ArF-meta (−94.2 (−96.8), ppm. IR (KBr): 3100–3000, 3000–2800, 2070, 1951, 1877, 1700, 1652, 1522, 1496, 1455, 1418, 1365, 1302, 1215, 1128, 1091, 962, 865, 761, 702, 549 cm⁻¹.

Preparation of PS-co-PPFS 25:75 (3)

DDMAT 1 (93.5 mg, 0.256 mmol), Sty (0.66 g, 6.4 mmol), PFS (3.73 g, 19.2 mmol), AIBN (4.2 mg, 26 μmol), and 1,4-dioxane (2.26 g, 25.6 mmol) were allowed to react according to the general polymerization procedure outlined for 2. The polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford 3 (1.4 g, yield 91%, conversion Sty 58%, conversion PFS 35%) as a pale yellow powder.

Preparation of PtBA (4)

A flame-dried 50 mL Schlenk flask equipped with a magnetic stir bar was charged with DDMAT 1 (406 mg, 1.12 mmol), tBA (20.0 g, 156 mmol), AIBN (9.2 mg, 56 μmol), and 2-butanol (11.3 g, 156 mmol). The flask was sealed with a rubber septum and stirred for 10 min at room temperature to ensure a homogeneous mixing. The reaction mixture was degassed by several freeze-pump-thaw cycles (>3), after which the flask was allowed to return to room temperature and was stirred for an additional 10 min. The flask was then immersed into a preheated oil bath at 60 °C to start the polymerization. The polymerization was monitored by analyzing aliquots collected at predetermined times by 1H NMR spectroscopy. As the expected monomer conversion was reached, after about 19 h, the polymerization was quenched by quick immersion of the reaction flask into liquid N₂. THF (15 mL) was added to the reaction flask and the polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford 4 (1.1 g, yield 92%, conversion Sty 30%, conversion PFS 49%) as a pale yellow powder.
pump-thaw cycles (>3), after which the flask was allowed to return to room temperature and was stirred for an additional 10 min. The flask was then immersed into a preheated oil bath at 60 °C to start the polymerization. The polymerization was monitored by analyzing aliquots collected at predetermined times by 1H NMR spectroscopy. As the expected monomer conversion was reached, after about 4.5 h, the polymerization was quenched by quick immersion of the reaction flask into liquid N2. THF (20 mL) was added to the reaction flask and the polymer was purified by precipitation into 2 L of a methanol/ice mixture thrice. The precipitants were collected and dried under vacuum overnight to afford 4 (12.6 g, yield 74%, conversion 68%) as a yellow powder.

1H NMR: $M_n = 13,200$ Da. GPC RI: $M_n = 11,900$ Da, $M_w = 12,800$ Da, PDI = 1.07. GPC LS: $M_n = 20,300$ Da, $M_w = 21,300$ Da, PDI = 1.05. 1H NMR (CDCl3): $\delta$ 0.86 (m, CH3CH2-), 1.14–1.85 (br, $-CHCH_2-$ of the polymer backbone, alkyl chain of initiator, and HOOC(CH3)2-), 1.36–1.56 (br, CH2C), 2.14–2.37 (br, $-CHCH_2-$ of the polymer backbone), 3.25–3.38 (br, $-SCSCH_2-$), 4.62–4.72 (br, $-CH_2CHS$) ppm. IR (KBr): 3000–2800, 1734, 1457, 1391, 1257, 1148, 1033, 962, 845 cm⁻¹.

Preparation of PtBA-b-(PS-co-PPFS) 75:25; General Procedure for the Block Extension (5)

A flame-dried 25 mL Schlenk flask equipped with a magnetic stir bar was charged with PtBA 4 (2.0 g, 0.15 mmol), Sty (0.40 g, 3.9 mmol), PFS (2.24 g, 11.5 mmol), AIBN (2.5 mg, 15 μmol), and 1,4-dioxane (1.36 g, 15.4 mmol) were reacted according to the general block extension procedure outlined in 5. The polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford 5 (2.2 g, yield 81%, conversion Sty 46%, conversion PFS 25%) as a yellow powder.

1H NMR: $M_n = 21,000$ Da. GPC RI: $M_n = 21,100$ Da, $M_w = 23,300$ Da, PDI = 1.16. GPC LS: $M_n = 18,600$ Da, $M_w = 21,800$ Da, PDI = 1.17. 1H NMR (CD2Cl2): $\delta$ 0.91 (m, CH3CH2-), 1.03–2.58, 2.59–2.92 (br, $-CHCH_2-$ and $-CHCH_2-$ of the polymer backbone, $-CH_2C$, alkyl chain of initiator, and HOOC(CH3)2-), 3.25–3.39 (br, $-SCSCH_2-$), 4.61–4.74 (br, $-CH_2CHS$), 6.37–7.38 (br, Ar–H) ppm. 19F NMR (CD2Cl2 relative to 1,1,2-trichloro-1,1,2-trifluoroethane): $\delta$ ArF-ortho (−74.4–(−75.4), ArF-meta (−87.4–(−92.0), ArF-meta (−94.2–(−96.8) ppm. IR (KBr): 3100–3000, 3000–2800, 1734, 1654, 1602, 1522, 1499, 1457, 1368, 1257, 1148, 1033, 962, 908, 846, 758, 700, 545, 471, 428 cm⁻¹.

Preparation of PtBA-b-(PS-co-PPFS) 25:75 (6)

PtBA 4 (2.0 g, 0.15 mmol), Sty (0.40 g, 3.9 mmol), PFS (2.24 g, 11.5 mmol), AIBN (2.5 mg, 15 μmol), and 1,4-dioxane (1.36 g, 15.4 mmol) were reacted according to the general block extension procedure outlined in 5. The polymer was purified by precipitation into methanol (3 × 500 mL). The precipitants were collected and dried under vacuum overnight to afford 6 (2.2 g, yield 81%, conversion Sty 46%, conversion PFS 25%) as a yellow powder.

1H NMR: $M_n = 21,000$ Da. GPC RI: $M_n = 21,100$ Da, $M_w = 23,300$ Da, PDI = 1.16. GPC LS: $M_n = 18,600$ Da, $M_w = 21,800$ Da, PDI = 1.17. 1H NMR (CD2Cl2): $\delta$ 0.91 (m, CH3CH2-), 1.03–2.58, 2.59–2.92 (br, $-CHCH_2-$ and $-CHCH_2-$ of the polymer backbone, $-CH_2C$, alkyl chain of initiator, and HOOC(CH3)2-), 3.25–3.39 (br, $-SCSCH_2-$), 4.61–4.74 (br, $-CH_2CHS$), 6.37–7.38 (br, Ar–H) ppm. 19F NMR (CD2Cl2 relative to 1,1,2-trichloro-1,1,2-trifluoroethane): $\delta$ ArF-ortho (−74.4–(−75.4), ArF-meta (−87.4–(−92.0), ArF-meta (−94.2–(−96.8) ppm. IR (KBr): 3100–3000, 3000–2800, 1734, 1654, 1602, 1522, 1499, 1457, 1368, 1257, 1148, 1033, 962, 908, 846, 758, 700, 545, 471, 428 cm⁻¹.
was added to the stirred solution and the reaction was stirred for 20 h at room temperature, after which the solvent was removed under vacuum. The crude product was resuspended in 15 mL of THF and transferred to a presoaked dialysis tubing (MWCO ca. 6000–8000 Da), and dialyzed against nanopure H₂O for 4 days to remove all of the impurities and afford polymer 9 as a white solid after lyophilization (728 mg, 93%, 2 mPEG/polymer chain, 40% coupling efficiency).

¹H NMR: \( M_n = 17,800 \) Da. ¹H NMR (DMSO-d₆): \( \delta = 0.92 \) (br, \( CH_3CH_2^- \)), 1.03–2.01, 2.05–2.37 (br, \(-CHCH_2^-\) and \(-CHCH_2^-\) of the polymer backbone, alkyl chain of initiator, and HOOC\((CH_3)₂CO^-\), 6.22–7.28 (br, \( Ar^-H \)), 11.88–12.45 (br, \(-COOH\)) ppm. ¹⁹F NMR (DMSO-d₆ relative to TFA): \( \delta = ArF\text{-ortho} (-65.0\text{--}(-68.0)), ArF\text{-para} (-82.0\text{--}(-85.0), ArF\text{-meta} (-87.1\text{--}(-90.2)) \) ppm. IR (KBr): 3500–2500, 1732, 1545, 1355, 1294, 1152, 1101, 955, 843, 757, 700, 667, 536 cm⁻¹.

**Preparation of PAA-g-(mPEG)-b-(PS-co-PPFS) 25:75 (10)**

Compound 8 (600 mg, 38.9 μmol), 10 mL DMF, HOBt (53 mg, 200 μmol), EDCI (75 mg, 200 μmol), and MeO-PEG₂₅₄NH₂ (389 mg, 195 μmol, 5 eq.) were allowed to react according to the general procedure outlined in 7 to afford polymer 8 as a white solid after lyophilization (730 mg, 88%, 3 mPEG/polymer chain, 60% coupling efficiency).

¹H NMR (DMSO-d₆): \( \delta = 0.90 \) (br, \( CH_3CH_2^- \)), 1.03–2.03, 2.07–2.40 (br, \(-CHCH_2^-\) and \(-CHCH_2^-\) of the polymer backbone, alkyl chain of initiator, and HOOC\((CH_3)₂CO^-\), 6.30–7.32 (br, \( Ar^-H \)), 11.90–12.56 (br, \(-COOH\)) ppm. ¹⁹F NMR (DMSO-d₆ relative to TFA): \( \delta = ArF\text{-ortho} (-64.2\text{--}(-70.4), ArF\text{-para} (-79.2\text{--}(-87.1), ArF\text{-meta} (-87.2\text{--}(-92.0)) \) ppm. IR (KBr): 3500–2500, 1732, 1651, 1520, 1455, 1355, 1167, 961, 866, 803, 761, 702, 652, 536 cm⁻¹.

**General Procedure for the Preparation of the Micelles (11)**

To a solution of diblock copolymer 9, in DMF (50 mg, 1.0 mg mL⁻¹), an equal volume of nanopure H₂O was added dropwise via a syringe pump over the course of 5 h. The reaction mixture was further stirred about 16 h at room temperature before being transferred to presoaked dialysis tubing (MWCO ca. 6000–8000 Da), and dialyzed against nanopure H₂O for 4 days to afford a micelle solution 11, with the final polymer concentration of 0.255 mg mL⁻¹; \( D_{h|b} \) (DLS) = 18 ± 2 nm; \( D_{h|vol} \) (DLS) = 23 ± 3 nm; \( D_{h|hint} \) (DLS) = 51 ± 6 nm;
$H_{av}$ (AFM) = 5 ± 1 nm. $^{19}$F NMR (D$_2$O relative to TFA + 10% (vol) DMSO): $\delta$ no signal detected.

**Preparation of Micelle (12)**

Polymer 10 was assembled into micelle 12 according to the general procedure outlined in 11, to give a micelle with a final concentration of 0.240 mg mL$^{-1}$. ($D_{h_{\text{av}}}$)$_n$ (DLS) = 17 ± 2 nm; ($D_{h_{\text{av}}}$)$_v$ (DLS) = 28 ± 4 nm; ($D_{h_{\text{av}}}$)$_{\text{int}}$ (DLS) = 116 ± 10 nm; $H_{av}$ (AFM) = 6 ± 1 nm. $^{19}$F NMR (D$_2$O relative to TFA + 10% (vol) DMSO): $\delta$ no signal detected.

**General Procedure for the Preparation of the SCKs (13)**

A solution of 2,2’-(ethylenedioxy)bis(ethylamine) in nanopure H$_2$O (ca. 1.0 mg mL$^{-1}$, nominal 50% crosslinking, 19.2 mg, 130 $\mu$mol) was added dropwise over 15 min to a solution of micelle 11 in nanopure H$_2$O (150 mL, 0.255 mg mL$^{-1}$ polymer concentration). The reaction mixture was stirred for about 1 h at room temperature. To this solution, a solution of 1-[3’-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide in nanopure H$_2$O (32 mg, 140 $\mu$mol) was added dropwise via syringe pump over the course of 4 h. The reaction mixture was further stirred for 20 h at room temperature before being transferred to presoaked dialysis tubing (MWCO ca. 6000–8000 Da), and dialyzed against nanopure H$_2$O for 4 days to remove all of the impurities and afford the SCK solution 13 with a final concentration of 0.25 mg mL$^{-1}$. ($D_{h_{\text{av}}}$) (DLS) = 26 ± 2 nm; ($D_{h_{\text{av}}}$)$_v$ (DLS) = 48 ± 5 nm; ($D_{h_{\text{av}}}$)$_{\text{int}}$ (DLS) = 136 ± 4 nm; $H_{av}$ (AFM) = 32 ± 4 nm. $^{19}$F NMR (D$_2$O relative to TFA + 10% (vol) DMSO): $\delta$ no signal detected.

**Preparation of the SCKs (14)**

Micelle 12 was crosslinked into SCK 14 according to the general procedure outlined in 13, to give a SCK with a final concentration of 0.238 mg mL$^{-1}$. ($D_{h_{\text{av}}}$)$_n$ (DLS) = 22 ± 3 nm; ($D_{h_{\text{av}}}$)$_v$ (DLS) = 32 ± 3 nm; ($D_{h_{\text{av}}}$)$_{\text{int}}$ (DLS) = 85 ± 10 nm; $H_{av}$ (AFM) = 20 ± 3 nm. $^{19}$F NMR (D$_2$O relative to TFA + 10% (vol) DMSO): $\delta$ no signal detected.

**General Procedure for the Loading of Perfluoro-15-crown-5-ether into the SCKs (15–20)**

A solution of PFCE (13.5 mg mL$^{-1}$ in CH$_2$Cl$_2$, 50% w/w with respect to the SCK) was added to a vial containing a magnetic stir bar and the SCK solution (4 mL, polymer concentration ~0.25 mg mL$^{-1}$). The solution was protected from light and stirred without a cap in a well-ventilated fume hood for 16 h to evaporate the CH$_2$Cl$_2$. The solution was then transferred to a centrifugal filter device (Amicon Ultra 4, 30 kDa MWCO, Millipore Corp., Billerica MA) and washed extensively with 5 mM PBS pH 7.4 buffer at 37 °C to remove unincorporated PFCE. The PFCE-nanoparticle solution was then reconstituted to a final volume of 4 mL. $^{19}$F NMR (D$_2$O relative to TFA): $\delta$ no signal detected.

**RESULTS AND DISCUSSION**

**Preparation of the Copolymers**

As a first step, two copolymers of Sty and 2,3,4,5,6-PFS were prepared to investigate the kinetics of the copolymerization of these monomers. The copolymers were prepared according to Scheme 1, by RAFT copolymerization in 1,4-dioxane solution using two different feed ratios of Sty and PFS with AIBN as radical initiator and DDMAT as chain transfer agent (CTA). As depicted in Figure 1 (top) and summarized in Table 1 (entries 1–2), polymers 2 and 3 showed unimodal and narrow molecular weight distributions (polydispersity indices (PDI) <1.2), as measured by GPC, indicating good control over the copolymerization process. There was agreement between the molecular weight values obtained by GPC and those determined by $^1$H NMR spectroscopy. $^1$H NMR spectroscopy also allowed for monitoring of the consumption of the two monomers independently, because of the unique shifts of their vinyl protons. A unique and opposite
preferential incorporation of Sty versus PFS monomers was observed for the two polymerizations that employed a majority of PFS versus Sty monomers, respectively, in the feed. For the copolymerization with a monomer feed of 75:25 mol % Sty/PFS, it was found that the conversion of Sty was only 30%, whereas 49% of PFS had undergone polymerization, after 19 h. The much faster propagation of the PFS monomer indicates that the polymer forms a tapered copolymer (PPFS-tap-PS), with the majority of the PFS consumed early during the polymerization. Still, the resulting polymer was comprised predominantly of Sty repeat units. In the case of the reversed monomer feed (25:75 Sty/PFS), the conversion was 58% for Sty and only 35% for PFS, suggesting that the polymer obtained is a tapered copolymer with the opposite connectivity (PS-tap-PPFS), again, though having an overall dominance of PFS in the polymer composition.

**Synthesis of the Block Copolymers**

The amphiphilic block copolymers in this study were prepared in a four-step fashion outlined in Scheme 1, by the copolymerization of Sty and PFS from a macro CTA (macroCTA). We hypothesized that having a high $T_g$ and fluorine-rich hydrophobic chain segment would help to increase the loading capacity of small molecule fluorinated components in the corresponding shell crosslinked nanoparticle core domain. The macroCTA of tBA was prepared by RAFT polymerization of tBA in 2-butane with DDMAT as the CTA, followed by chain extension with different ratios of Sty and PFS to yield well-defined block copolymers 5 and 6. As in the case of the copolymers described in the previous section, the copolymerization of Sty and PFS resulted in similarly tapered monomer consumptions, with higher PFS conversion for the copolymer with low PFS feed. GPC characterization of the block copolymers indicated a clear molecular weight increase from the macroCTA after chain extension with Sty and PFS (Fig. 1 (bottom)). The PDIs of the block copolymers were low ($<1.2$); however, a small tail toward lower molecular weight can also be seen for polymer 6. Both block copolymers had molecular weights in fair agreement with the theoretical values (Table 1) indicating good control over the polymerization. The amphiphilic block
copolymers were prepared by the removal of the tert-butyl group of the PrBA by acidolysis with TFA in CH$_2$Cl$_2$ to yield the PAA-b-(PS-tap-PPFS) 7 and PAA-b-(PPFS-tap-PS) 8 block copolymers.

Because of our interest, ultimately, in the application of the final materials in medical applications and because systemically administered nanoparticles tend to be sequestered by the mononuclear phagocyte system (also referred to as the reticuloendothelial system), and rapidly accumulate mainly in the liver and spleen, it is necessary to increase the blood circulation time of these materials if they are to be used in vivo. The biodistribution can be altered by attaching PEG chains to the diblock copolymer. For this purpose, 2 kDa monomethoxy-terminated PEG (mPEG) chains were grafted by amidation onto the acrylic acid segments of the block copolymer backbones of 7 and 8, respectively, to yield polymer 9 with approximately two mPEG grafts per polymer chain and polymer 10 with three mPEG grafts per polymer chain. These polymers were then further self-assembled into micelles and SCKs.

**Preparation of the SCKs**

As depicted in Scheme 2, micelles from the PAA-g-PEG-b-(PS-co-PPFS) block copolymers 9 and 10 were prepared by following the protocol described in the experimental section to result in polymeric micelles 11 and 12. The SCKs were obtained by intramicellar crosslinking of their hydrophilic shell layers through condensation reactions, nominally, upon 50% of the carboxylic acid functionalities of the PAA segments with a diamino crosslinker (2,2$^\prime$-(ethylenedioxy) bis(ethylamine)) to yield SCKs 13 and 14. The crosslinking reactions were followed by extensive dialysis against nanopure water to remove the small molecule byproducts.

**Characterization of the SCKs**

The shapes and sizes of micelles 11 and 12 and SCKs 13 and 14 were investigated by AFM (Fig. 2) and DLS. The results of these characterizations are summarized in Table 2. AFM imaging on mica was employed to characterize the micelles and SCKs. The micelles 11 and 12 were observed to flatten on the mica substrate and were found to have average heights of about 8 and 7 nm, respectively. Crosslinking to afford SCKs 13 and 14 resulted in more shape-persistent nanoparticles that flattened less on the substrate, giving an average height of about 32 nm for SCK 13 and about 20 nm for SCK 14.

The micelles and SCKs were also analyzed by DLS to study the size of the particles in solution (Table 2). The micelles ranged in size between 17 and 18 nm (hydrodynamic diameter by number) and the SCKs between 22 and 26 nm. Micelle 12 and SCKs 13 and 14 had larger intensity-average hydrodynamic diameter values, in comparison to the number- and volume-based averages, indicating the presence of a small portion of aggregated micelles of larger size (see DLS histograms in the Supp. Info.).

**Figure 1.** GPC chromatograms of the copolymers 2–3 (upper panel) and the block copolymers 4–6 (lower panel).
Figure 3 depicts the $^{19}$F NMR spectra of the polymers prepared, in which it can be seen that the resonances corresponding to the ortho, meta, and para aromatic fluorines of the PFS are broad signals with line widths exceeding 300 Hz. When the tert-butyl groups of the polymer are removed (polymer 7) and PEG chains are grafted (polymer 9), the line width increases further, and the signals shift by about 15 ppm, because of the change from CD$_2$Cl$_2$ to DMSO as NMR solvent. When these polymers were assembled into polymeric micelles (micelle 21, Fig. 3), the signal corresponding to the fluorinated backbone completely disappeared, indicating a significant reduction of mobility in the core domain, which has been seen for other fluorinated polymer assemblies. To ensure that the signal from the polymer did not shift beyond the expected shift region, these NMR experiments were run with very broad spectral width (1200 ppm) and over extended periods of time (6000 scans) with long delay times.

**Table 1. Summary of Data for the Polymers Prepared**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Sample</th>
<th>$M_n$ (Da)$^a$</th>
<th>$M_w$ (Da)$^b$</th>
<th>$M_D$ (Da)$^b$</th>
<th>PDI$^b$</th>
<th>$M_n$ (Da)$^c$</th>
<th>$M_w$ (Da)$^c$</th>
<th>PDI$^c$</th>
<th>DP</th>
<th>DP</th>
<th>mPEG$^e$</th>
<th>$T_g$ ($^\circ C$)$^f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>PS-co-PPFS</td>
<td>5,100</td>
<td>5,500</td>
<td>6,100</td>
<td>1.10</td>
<td>4,800</td>
<td>4,900</td>
<td>1.02</td>
<td>23</td>
<td>12</td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>PS-co-PPFS</td>
<td>7,000</td>
<td>6,800</td>
<td>7,400</td>
<td>1.09</td>
<td>8,500</td>
<td>8,600</td>
<td>1.02</td>
<td>15</td>
<td>26</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>4</td>
<td>PtBA$_{100}$</td>
<td>13,200</td>
<td>11,900</td>
<td>12,800</td>
<td>1.07</td>
<td>20,300</td>
<td>21,300</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td>41</td>
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<td>5</td>
<td>PtBA-b-(PS-co-PPFS)</td>
<td>19,000</td>
<td>22,100</td>
<td>26,200</td>
<td>1.18</td>
<td>14,700</td>
<td>16,500</td>
<td>1.18</td>
<td>30</td>
<td>14</td>
<td></td>
<td>52, 82</td>
</tr>
<tr>
<td>6</td>
<td>PtBA-b-(PS-co-PPFS)</td>
<td>21,000</td>
<td>21,100</td>
<td>23,300</td>
<td>1.16</td>
<td>18,600</td>
<td>21,800</td>
<td>1.17</td>
<td>19</td>
<td>30</td>
<td></td>
<td>56, 88</td>
</tr>
<tr>
<td>7</td>
<td>PAA-b-(PS-co-PPFS)</td>
<td>13,800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>87</td>
</tr>
<tr>
<td>8</td>
<td>PAA-b-(PS-co-PPFS)</td>
<td>15,400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>79</td>
</tr>
<tr>
<td>9</td>
<td>PAA-g-PEG-b-(PS-co-PPFS)</td>
<td>17,800</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>PAA-g-PEG-b-(PS-co-PPFS)</td>
<td>21,400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

$^a$ Calculated from $^1$H NMR spectroscopy.
$^b$ RI GPC.
$^c$ LS GPC.
$^d$ Calculated from $^1$H NMR spectroscopy.
$^e$ Calculated from $^1$H NMR spectroscopy.
$^f$ Determined from DSC (see Supp. Info.).
$^g$ Unsuitable for GPC analysis with THF as the eluent.

$^{19}$F NMR Characterization of the Polymers, Micelles, and SCKs

Figure 3 depicts the $^{19}$F NMR spectra of the polymers prepared, in which it can be seen that the resonances corresponding to the ortho, meta, and para aromatic fluorines of the PFS are broad signals with line widths exceeding 300 Hz. When the tert-butyl groups of the polymer are removed (polymer 7) and PEG chains are grafted (polymer 9), the line width increases further, and the signals shift by about 15 ppm, because of the change from CD$_2$Cl$_2$ to DMSO as NMR solvent. When these polymers were assembled into polymeric micelles (micelle 21, Fig. 3), the signal corresponding to the fluorinated backbone completely disappeared, indicating a significant reduction of mobility in the core domain, which has been seen for other fluorinated polymer assemblies. To ensure that the signal from the polymer did not shift beyond the expected shift region, these NMR experiments were run with very broad spectral width (1200 ppm) and over extended periods of time (6000 scans) with long delay times.

**Loading of Perflouro-15-crown-5-ether**

To utilize these nanoparticle constructs for MRI applications, higher fluorine concentrations are necessary than those provided by the PPFS units and, therefore, a procedure similar to the one developed for incorporating doxorubicin into SCKs (Scheme 2 middle) was explored for incorporating PFCE into the SCK cores. We selected PFCE because it is a small molecule with high fluorine ratio and 20 NMR-equivalent $^{19}$F atoms. A dilute PFCE solution in DCM was added to a stirred SCK solution at nominal loading levels of 50, 100, and 200% of the polymer mass. DCM acted as a selective solvent for the hydrophobic core, facilitating the incorporation of PFCE in the core domain. After the solvent was evaporated, the nanoparticle solutions were purified by several washings using a centrifugal filter (MWCO 30 kDa), to yield transparent nanoparticle solutions. The filtrate was analyzed for nonencapsulated PFCE by $^{19}$F NMR, which was not detected. However, no PFCE was detectable in any of the SCKs either, regardless of the relative loading percentages, even after extended $^{19}$F NMR experiments (5000 scans). To test whether the restricted mobility provided by the glassy PPFS/PS hydrophobic core was limiting the possibility to see the signals arising from PFCE, the samples were loaded with 10% DMSO (by volume), which acted as a solvent for both core and shell of the nanoparticle. Unfortunately, no signals from either the PPFS core or the PFCE were detectable by $^{19}$F NMR spectroscopy. From this result, one can draw two conclusions: (1) that the loading procedure was not suitable for high surface tension liquids, such as PFCE, and/or (2) that the mobility of the fluorinated polymer in the SCK core was highly restricted and, thereby, prohibiting $^{19}$F NMR spectroscopy.
NMR spectroscopy. A simple mixing of PFCE with the SCK without the aid of an organic solvent did not result in any PFCE incorporation, most likely because of the PFCE phase separating from the SCK solution, increasing the surface tension to the extent that a small magnetic stir bar would float in the PFCE-SCK interface. The restricted mobility is also apparent from the DSC runs on the lyophilized SCKs, for which the \( T_g \) increases from about 75 °C for the starting copolymer 9 (PS-co-PPFS) to about 95 °C for the assembled and crosslinked SCK 13. A similar trend was observed for SCK 14, which derived from 10 (See Supp. Info.).

Instead, a comicellization procedure was elaborated, in which polymer 9 and PFCE (50% by weight) were mixed in DMF before the slow addition of nanopure water (Scheme 2) to induce self-
assembly to polymer micelles. The micelles were subsequently dialyzed for 4 days to remove the unincorporated PFCE, and were found to have a hydrodynamic diameter of 20 ± 3 nm, similar in size to the neat polymer micelle based on polymer 9. The micelle was further analyzed by 19F NMR (Fig. 3), showing no signals except for the TFA reference. This sample was then also loaded with DMSO and, as seen in Figure 3, a signal corresponding to PFCE at 6 ppm could then be detected. This peak is shifted somewhat compared with PFCE in D2O DMSO that resonates at about 13 ppm, relative to TFA (image not shown). This shift is indicative that the local environment affects the PFCE, even though the polymer core has been swollen with DMSO. In all, this study further suggests that the fluorinated core restricts the mobility of small molecule guests confined therein, but that addition of a core selective solvent allows for the detection of the PFCE guests by 19F NMR. However, importantly, confirmation was made that this loading process during micellization was effective. The loading of PFCE was then calculated by 19F NMR spectroscopy, integrating the peak corresponding to PFCE, and comparing it to the TFA that was used as internal standard (Fig. 3). The loading percentage was 9% (mass % polymer) for the PFCE-micelle based on polymer 9, corresponding to a fluorine concentration of 0.82 mM and a loading effectiveness of 8%.

CONCLUSIONS

Reversible addition-fragmentation chain transfer (RAFT) polymerization was employed to prepare copolymers of PS and PPFS, as well as block copolymers of PrBA-b-PS-co-PPFS with good control over molecular weight and polydispersity. It was found that the copolymerization of Sty and PFS with a 75:25 mol % feed ratio results in a greater proportion of consumption of the minor PFS monomer, to afford copolymers of a gradient

## Table 2. Summary of Data for Micelles and SCKs Prepared

<table>
<thead>
<tr>
<th>Sample</th>
<th>Conc. (mg mL(^{-1}))</th>
<th>((D_h)_n) (nm)(^a)</th>
<th>((D_h)_\text{vol}) (nm)(^a)</th>
<th>((D_h)_\text{int}) (nm)(^a)</th>
<th>(H_{av}) (nm)(^b)</th>
<th>Conc. ((%) of poly)</th>
<th>PFCE (1) (^{19})F Conc. (mM)</th>
<th>Experimental (1) (^{19})F Conc. (mM)</th>
<th>(T_g) ((^°)C)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>0.255</td>
<td>18 ± 2</td>
<td>23 ± 3</td>
<td>51 ± 6</td>
<td>8 ± 1</td>
<td>0%</td>
<td>1(^d)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>0.240</td>
<td>17 ± 2</td>
<td>28 ± 4</td>
<td>116 ± 10</td>
<td>7 ± 1</td>
<td>0%</td>
<td>1.7(^d)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>0.25</td>
<td>26 ± 2</td>
<td>48 ± 5</td>
<td>136 ± 4</td>
<td>32 ± 4</td>
<td>0%</td>
<td>0.98(^d)</td>
<td>Not detected</td>
<td>98</td>
</tr>
<tr>
<td>14</td>
<td>0.238</td>
<td>22 ± 3</td>
<td>32 ± 3</td>
<td>84 ± 10</td>
<td>20 ± 3</td>
<td>0%</td>
<td>1.7(^d)</td>
<td>Not detected</td>
<td>97</td>
</tr>
<tr>
<td>15(^e)</td>
<td>0.25</td>
<td>31 ± 3</td>
<td>87 ± 10</td>
<td>202 ± 11</td>
<td>–</td>
<td>50%</td>
<td>6.5(^f)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>16(^e)</td>
<td>0.24</td>
<td>25 ± 5</td>
<td>39 ± 9</td>
<td>178 ± 10</td>
<td>–</td>
<td>100%</td>
<td>12.9(^f)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>17(^e)</td>
<td>0.25</td>
<td>24 ± 3</td>
<td>51 ± 10</td>
<td>189 ± 12</td>
<td>–</td>
<td>200%</td>
<td>25.9(^f)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>18(^f)</td>
<td>0.24</td>
<td>37 ± 4</td>
<td>155 ± 20</td>
<td>236 ± 10</td>
<td>–</td>
<td>50%</td>
<td>6.2(^f)</td>
<td>Not detected</td>
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<tr>
<td>19(^f)</td>
<td>0.25</td>
<td>44 ± 4</td>
<td>188 ± 22</td>
<td>227 ± 17</td>
<td>–</td>
<td>100%</td>
<td>12.3(^f)</td>
<td>Not detected</td>
<td>–</td>
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<tr>
<td>20(^f)</td>
<td>0.24</td>
<td>54 ± 4</td>
<td>270 ± 13</td>
<td>245 ± 10</td>
<td>–</td>
<td>200%</td>
<td>24.6(^f)</td>
<td>Not detected</td>
<td>–</td>
</tr>
<tr>
<td>21</td>
<td>0.26</td>
<td>20 ± 3</td>
<td>31 ± 6</td>
<td>158 ± 12</td>
<td>–</td>
<td>50%</td>
<td>5.1(^f)</td>
<td>0.82</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^{a}\)From DLS.
\(^{b}\)From AFM.
\(^{c}\)Determined from DSC (see Supp. Info.).
\(^{d}\)[\(^{19}\)F] originating from PPFS.
\(^{e}\)From SCK 13.
\(^{f}\)[\(^{19}\)F] originating from PFCE.
\(^{g}\)From SCK 14.
sequence, PPFS-grad-PS, having decreasing relative PFS along the backbone as the conversion proceeded, but with a majority of Sty units overall. An opposite trend was observed when the feed ratio was reversed. This interesting copolymerization behavior was observed for both neat copolymerization of Sty and PFS, and also for the block extensions from a PrBA macroCTA.

The block copolymers were converted into amphiphilic PAA-b-(PS-co-PPFS), fitted with mPEG grafts and, subsequently, assembled into polymeric micelles and SCK nanoparticles of small size, about 25 nm, in aqueous solution. It was found that the assembly of the polymers into core-shell micelles and SCKs restricted the mobility of the fluorinated core domain, which then limited their $^{19}$F NMR signal and their use as MRI contrast agents. Softening of the core of the SCK by the addition of DMSO did not improve the mobility to the extent that the polymer signals could be observed, suggesting that the glassy amorphous core of the SCK ($T_g$ of 97 $^\circ$C) restricted mobility. Additionally, loading of PFCE as a small molecule $^{19}$F label within the SCKs in aqueous solution in a postpreparation loading process gave either insufficient loading or insufficient mobility to allow for the PFCE to be observed by $^{19}$F NMR spectroscopy. In contrast, by a comicellization procedure beginning from neat DMF as an organic cosolvent, PFCE could be loaded into the polymer micelle with good loading capacity and the $^{19}$F NMR signal could be observed after swelling with DMSO. To solve the problem of the restricted mobility in the core, which prohibits the use of these nanoparticles as MRI agents, polymers with low $T_g$ and high fluorine content must be developed.

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![Figure 3. $^{19}$F NMR spectra of the copolymer 2 in CD$_2$Cl$_2$, block copolymer 5 in CD$_2$Cl$_2$, amphiphilic block copolymer 7 in DMSO-d$_6$, PEG-grafted amphiphilic block copolymer 9 in DMSO-d$_6$, PCFE-loaded micelle 21 in D$_2$O, PCFE-loaded micelle 21 in D$_2$O with 10% DMSO.](image)
Excellence in Nanotechnology (HL080729) and by the Office of Naval Research (N00014-08-1-0398). Postdoctoral and assistant professor fellowship provided by the Knut and Alice Wallenberg Foundation is gratefully acknowledged (A. M. Nyström). The authors are grateful for Jeff Kao’s assistance with $^{19}$F NMR and $T_1/T_2$ measurements.

REFERENCES AND NOTES


