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Multicompartment Theranostic Nanoemulsions Stabilized by a Triphilic Semifluorinated Block Copolymer

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Abstract

The presence of a perfluorocarbon block in a multiblock polymer has been shown to be an additional driving force toward nanoparticle assembly. In the preparation of nanoemulsions, this perfluorocarbon block also provides enhanced particle stability. Herein, the synthesis of a new triphilic, semifluorinated copolymer, M2F8H18, is introduced. This ABC type block copolymer can be used to formulate extremely stable nanoemulsions, assembled around a lipophilic droplet, with lifetimes of one year or more. The central oil droplet can stably solubilize high concentrations of hydrophobic drugs, making this system an ideal drug delivery vehicle. The incorporation of the perfluorocarbon block modulates drug release from the lipophilic core via the surrounding fluororous shell. Fluorous imaging agents incorporated into the fluororous shell prolong drug release even further as well as provide potent ¹⁹F-MRI contrast ability. *In vitro* studies show that these nanoemulsions efficiently inhibit cancer cell growth, thus providing a theranostic drug delivery system.

Graphical Abstract:

Keywords

semifluorinated polymers; block copolymers; nanoemulsions; drug delivery; hydrophobic drugs; paclitaxel; ¹⁹F-MRI; theranostics

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Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: [10.1021/acs.molpharmaceut.7b00624](https://doi.org/10.1021/acs.molpharmaceut.7b00624). NMR and MS spectra, HPLC profiles, surface tension measurement plot, and DLS data (PDF)

Notes

The authors declare no competing financial interest.

INTRODUCTION

Copolymers that can form nanoscale assemblies in aqueous solution have vast potential in the field of drug delivery. Different polymer designs result in varied nanoparticle sizes, shapes, and properties, as desired by the specific application. Incorporation of a fluororous block into the polymer provides an enhanced driving force toward assembly and particle stability overall, in comparison to typical hydrocarbon polymers. This is a result of the unique properties of fluororous blocks which include both hydrophobicity and lipophobicity, high thermal and chemical stability, high surface tension, and biological inertness. In addition, due to the extremely strong carbon–fluorine bond (472 kJ/mol) and to the size of a carbon-bound fluorine atom (vdW radius = 1.47 Å) the fluororous blocks have very low polarizability and rigid, helical structures.^{1–3} These properties result in the formation of an energetically favored fluororous phase in solution and, with it, increased particle stability. For these reasons, semifluorinated polymers are highly valuable for the design of drug delivery systems.

Colloidal nanoemulsions are kinetically stable nanoparticles in the biomedically useful size range of 100–400 nm.⁴ These particles can be formulated by the input of a high amount of energy into a solution of two immiscible liquids and an appropriate surfactant. The nanoemulsions described here are oil-in-water emulsions, in which a drug-containing oil droplet is stabilized in an aqueous environment by a shell of M2F8H18 surfactant. Nanoemulsions of this design are especially advantageous over other nanoparticle systems due to their large oil droplet core and their resulting ability to solubilize and deliver copious amounts of hydrophobic drugs. Furthermore, due to its particle size, this nanoscale system is able to exploit the enhanced permeability and retention (EPR) effect to preferentially accumulate within tumor tissue over healthy tissue due to leaky tumor vasculature and a poorly functioning tumor lymphatic system.^{5–7} This type of selective delivery to tumor tissue, as well as the high drug payload afforded by nanoemulsions, makes them a potential therapeutic tool for use in cancer treatment.

Previous work in our laboratory with nanoemulsions has primarily focused on semifluorinated diblock copolymer based nanoemulsions for successful emulsification of fluororous anesthetics. Here, a new semifluorinated, triblock ABC copolymer is introduced consisting of hydrophilic, fluorophilic, and lipophilic segments that can form triphilic nanoemulsions.^{8–12} This innovative triphilic design is ideal not only for the delivery of solid chemotherapeutic drugs dissolved in the lipophilic core but also for use as a theranostic system by the addition of a fluororous imaging agent present in the intermediary fluororous shell. The introduction of the fluorocarbon block also allows for the development of nanoparticles that can modulate drug retention and exhibit enhanced kinetic stability. The unique properties of the triphilic polymer M2F8H18 (Figure 1) allow for high loading and retention of hydrophobic drugs within the oil droplet core. In the emulsion nanoparticles, the lipophilic block of the polymer penetrates the oil core, stabilizing the particles and interacting noncovalently with the drug and oil. Drug release from the core is then modulated by the thickness of the nanoparticle intermediate fluororous shell. Particle water solubility and biocompatibility are achieved through the methoxy capped poly(ethylene

glycol) (mPEG) hydrophilic block comprising the particle corona. The addition of a fluorine imaging agent provides further particle stability, enhanced drug retention, and potent ^{19}F magnetic resonance imaging (MRI) signal and imaging possibilities.

Drug-encapsulation and release properties of the nanoemulsions were tested using the chemotherapeutic paclitaxel. This antimitotic drug was selected for its high potency against many cancer types including breast, ovarian, and lung cancer as well as Kaposi's carcinoma. Paclitaxel functions by the promotion of tubulin polymerization and equimolar binding to tubulin dimers, resulting in mitotic arrest.^{13,14} Paclitaxel is highly hydrophobic with a water solubility $<1\text{ }\mu\text{g/mL}$ and exhibits poor *in vivo* pharmacokinetics. Due to these challenging characteristics, paclitaxel was first administered intravenously as Taxol in combination with Cremophor EL, a nonionic surfactant with polyoxyethylated castor oil, and ethanol. However, Taxol leads to widespread hypersensitivity reactions, plasticizer leaching, and even neuropathy due to the Cremophor EL surfactant and other solvents.¹⁵ An additional paclitaxel formulation, Abraxane, was approved in 2005 involving albumin-bound paclitaxel nanoparticles.¹⁶ Despite dramatic improvements in side effect toxicity, this formulation still lacks high potency due to the discrepancy between injected drug and drug that actually reaches the tumor.^{17,18} Thus, an improved delivery method for this highly cytotoxic drug is still needed. Due to the extremely poor solubility of paclitaxel in water and other solvents, an optimization was performed to determine what lipophilic medium could best carry this chemotherapeutic payload. Common oils were investigated including 2-octyl-1-dodecanol, soybean oil, glycerol, and their combinations. These media resulted in many issues including very low paclitaxel solubility, high oil viscosity, and bacterial growth within the oil. Further investigation led to the use of medium chain triglycerides (MCT), an FDA approved lipophilic medium mainly derived from coconut oil.¹⁹ Similar to soybean oil, MCT contains a triglyceride backbone, but in contrast to soybean oil, which contains both saturated and unsaturated fatty acids, MCT only contains fully saturated fatty acids. The MCT grade used here (Neobee M-5) contains a range of eight to ten carbon units per chain. Furthermore MCT has been used widely in nutritional supplements and drug formulations since this oil is easily hydrolyzed, metabolized, and absorbed, which results in its fast conversion to energy.^{20,21} Paclitaxel interacts favorably with MCT with formulations up to 15 mg of paclitaxel per mL of MCT reported herein. Due to its many advantageous properties, MCT was selected as the medium of choice to compose the lipophilic core of the M2F8H18 nanoemulsions.

Theranostics is a term describing dual therapeutic and diagnostic character. Theranostic nanomedicine is an expanding field geared toward personalized medicine that combines therapeutic and imaging treatments for a clear picture of drug distribution and efficacy. As such, theranostic agents frequently involve administration of a chemotherapy, gene therapy, or radiation therapy with an imaging agent.²² The described M2F8H18 nanoemulsions are formulated into a theranostic system upon combination of paclitaxel, the therapeutic portion, with a ^{19}F -MRI contrast agent, the diagnostic portion. Perfluoro-15-crown-5-ether (PFCE) was selected as the fluorine contrast agent to be used in these nanoemulsions. This clear, organic oil is very stable under physiological conditions with a high boiling point. The structure of the macrocycle is thought to tend toward a puckered ring with high flexibility in solution leading to all 20 fluorine atoms exhibiting a single peak in ^{19}F NMR.²³ The

presence of just a single resonance signal makes this system highly sensitive in ^{19}F NMR and therefore in ^{19}F -MRI applications. Previously, PFCE has found ^{19}F -MRI uses in labeling and tracking dendritic cells, stem cells, plaques, and even imaging sites of inflammation.^{24–30} The use of our semifluorinated M2F8H18 copolymer allows for the binding of very high concentrations of fluorophilic PFCE in the intermediate fluorophilic shell (Figure 1) thereby providing a sensitive imaging system with greater stability than a traditional surfactant.

The use of MRI for diagnostic purposes continues to expand due to its noninvasive nature, deep tissue penetration, excellent sensitivity, and widespread application. Most frequently, ^1H nuclei are utilized due to their high sensitivity of 1.0, natural abundance of 99%, and the large amount of water present in anatomical systems. Though less common, ^{19}F nuclei can be used easily for MRI with similar sensitivity to proton, 0.83, and an even higher natural abundance of 100%.²⁹ Furthermore, fluorine is not a naturally occurring biological element, and this leads to a near-zero background in ^{19}F -MRI as well as an excellent signal-to-noise ratio.^{30,31} In order to clearly visualize tissues in traditional ^1H -MRI, a contrast agent is commonly required; two of the most common include chelated gadolinium and superparamagnetic iron oxide (SPIO). Both metallic compounds are very efficient at indirect imaging but have exhibited several toxicity concerns including oxidative stress, altered gene expression, and, in extreme cases, kidney failure.³² In an analogous manner, fluorocarbons with high signal intensity can be used to image anatomical systems. In this case, however, the imaging can be performed directly. This work utilizes the intrinsic properties of ^{19}F nuclei via the incorporation of perfluoro-15-crown-5-ether. This excipient was selected due to its high signal intensity and reportedly low toxicity compared to other fluorinated crown ethers.³³

Described in this article are the synthetic methods and physicochemical characteristics of the M2F8H18 copolymer. The formulation of nanoemulsions stabilized by M2F8H18 will be discussed herein including their unprecedented long-term stability, *in vitro* drug release profiles, and *in vitro* and *in vivo* toxicity profiles. The theranostic potential of these nanoemulsions will also be presented through preliminary ^{19}F -MRI work.

EXPERIMENTAL SECTION

Materials and Methods.

1H,1H,10H,10H-Perfluorodecane-1,10-diol was purchased from SynQuest Laboratories Inc. (Alachua, FL), and perfluoro-15-crown-5-ether was purchased from Oakwood Chemical (Estill, SC). Normal saline (AirLife sterile 0.9% sodium chloride for irrigation USP) was purchased from the University of Wisconsin Hospital Pharmacy and paclitaxel from LC Laboratories (Woburn, MA). Solvents and all other reagents were purchased from Sigma-Aldrich Co. (Milwaukee, WI) and Spectrum (Gardena, CA) and used as received, unless otherwise mentioned. Small molecular and polymer chromatography was accomplished with Silicycle 60 Å SiO_2 or using a Teledyne CombiFlash Rf 4× (Lincoln, NE) equipped with an ELSD for visualization and REDi-sep Rf high performance silica or C18 columns.

^1H and ^{19}F NMR spectra were obtained on a Varian Unity-Inova 400 or Varian Unity-Inova 500 spectrometer using CDCl_3 as the solvent (unless otherwise specified) and TMS as the internal reference. Polymer purity was confirmed by HPLC with a Gilson 321 Pump (Middleton, WI) equipped with a Jordi Gel DVB 500 Å (Bellingham, MA) column and a Gilson Prep-ELS detector and by MALDI-MS on a Bruker Ultraflex III MALDI TOF/TOF using α -cyano-4-hydroxycinnamic acid matrix unless otherwise specified.

Critical Micelle Concentration (CMC): Surface Tensiometry.

Polymer was dissolved in Millipore Milli-Q water in a 20 mL disposable scintillation vial to the desired maximum concentrations (1.0 and 0.32 mM). The solutions were shaken and sonicated for 3 h. Solutions were then allowed to equilibrate for 24 h. Serial dilutions were then prepared from these two stock solutions to achieve the desired concentrations. Each serial dilution was also sonicated for 3 h and allowed to equilibrate prior to the next dilution. Once all solutions were prepared, they were allowed to equilibrate for an additional 24 h. Surface tensions were measured on a KSV sigma 701 tensiometer (KSV Instruments, Helsinki, Finland). A custom round platinum rod, with a diameter of 1.034 mm and a wetted length of 3.248 mm from KSV Instruments (Helsinki, Finland), was initially cleaned with ethanol and dried in a Bunsen burner flame. Before running the experimental samples, the surface tension of Millipore Milli-Q water was measured as control to confirm that the rod was fully cleaned and surface tension was within $\pm 2 \text{ mN m}^{-1}$ of the literature value, 78.2 mN m^{-1} . The surface tension of each sample was then measured using the Wilhelmy method, beginning with the least concentrated solution and proceeding to successively more concentrated solutions. The surface tension at each concentration was measured in quadruplet and the average recorded. The CMC value was determined from the intersection of the slope at the crossover point of two lines: the baseline of minimal surface tension and the slope where surface tension showed linear decline. Error was determined by weighted least-squares analysis.

Nanoemulsion Formulation.

Aqueous polymer solutions were prepared freshly (20 mM) in sterile, normal saline and sonicated at room temperature until fully dissolved. Saline was composed of 0.9% (w/w) sodium chloride.

Paclitaxel/medium chain triglycerides (MCT) solutions were prepared freshly. Two milliliters of paclitaxel solution (prepared at a concentration of 7.5 mg/mL in 50:50, acetonitrile:ethanol) was dissolved in MCT followed by heating and stirring until fully solubilized. All traces of acetonitrile and ethanol were removed by vacuum.

Paclitaxel/MCT solution and PFCE were added to the polymer solution. The homogenizer and microfluidizer were first cleaned with 100% and 70% ethanol followed by 100% and 70% methanol and finally three rinses with Millipore Milli-Q water to remove all traces of any previous nanoemulsions. The prepared mixture was then homogenized with the high-speed homogenizer (Power Gen 500, Fisher Scientific, Hampton, NH) for 1 min at 21,000 rpm at room temperature. The resulting crude emulsion was then further mixed with the microfluidizer (model M-110S, Microfluidics Corp., Newton, MA) for 1 min under 5,000

psi with the cooling bath kept at 0 °C. The final emulsion was then filtered with a 0.45 μm nylon filter and stored in a sterile, plastic centrifuge tube (Corning Inc., Corning, NY) at 4 °C.

Particle Size Determination via Dynamic Light Scattering (DLS).

Long-term nanoemulsion size and stability was monitored via dynamic light scattering (NICOMP 380ZLS, Particle Sizing Systems, Santa Barbara, CA). The nanoemulsions were diluted at the intensity factor of 500 by adding 5 μL of the nanoemulsion to 3.0 mL of Millipore Milli-Q water. Each particle size analysis was run for 5 min at room temperature in a quartz cuvette and repeated three times. The data was analyzed using Gaussian analysis and reported as intensity weighted average diameters.

Micellar aggregate size, distribution, and histogram data were determined via dynamic light scattering (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, U.K.). The nanoemulsions were diluted at the same ratio as above, 5 μL of nanoemulsion to 3.0 mL of Millipore Milli-Q water. Each particle analysis was run as a set of 10 scans in a semimicro polystyrene cuvette at room temperature and repeated three times. The data was analyzed using Malvern software analysis and reported as volume weighted average diameters.

In Vitro Drug Release.—The nanoemulsion was initially diluted by a factor of 20 (0.125 mL of nanoemulsion plus 2.375 mL of Millipore Milli-Q water). A time-zero time point was established by diluting 100 μL of diluted nanoemulsion mixture above in 900 μL of acetonitrile (ACN). A 3 mL capacity Slide-A-Lyzer Dialysis cassette (G2 2,000 MWCO from Thermo Fisher Scientific Inc., Fitchburg, WI) was hydrated by stirring for 12 h in a 3 L PBS bath (300 mL of 10 $\times$ PBS and 2,700 mL of Millipore Milli-Q water) at 37 °C. After this time, the remaining diluted nanoemulsion solution and 100 μL of PBS (1 $\times$) were added to the cassette, which was then returned to the PBS bath and stirred for 1 week at 37 °C; this was performed in triplicate. Time points were taken at 0.5, 2, 3, 6, 9, 12, 24, 36, 48, 72, 96, 120, 144, and 168 h. At each time point a long-stemmed glass pipet was used to mix the contents of the cassette three times. Then a 100 μL aliquot of nanoemulsion was removed from the cassette and diluted with 900 μL of ACN. The nanoemulsion aliquot was then replaced in the cassette by 100 μL of fresh PBS (1 $\times$). Sink conditions were maintained by replacing the 3 L PBS baths at the 3, 6, 9, and 12 h time points and every 12 h thereafter.

The paclitaxel concentration remaining in the nanoemulsion at each time point was quantified by reverse phase HPLC. The HPLC system used was a Shimadzu prominence HPLC system (Shimadzu, Japan) equipped with an LC-20AT pump, SIL-20 AC HT autosampler, CTO-20 AC column oven, and an SPD-M20A diode array detector. For each time point sample, 20 μL was injected into a C18 column (Agilent XDB-C18, 4.6 $\text{\AA}$ $\times$ 150 mm) and eluted with an isocratic mixture of 25% water and 75% ACN. The run time was 7 min, the flow rate was 1.0 mL min⁻¹, and the detection was set at 227 nm. Paclitaxel eluted at 4.15 min. Concentration of paclitaxel was determined by integrating the area of the peak and extrapolation from a standard calibration curve (500, 100, 50, 25, 10, 5, 2.5 $\mu\text{g mL}^{-1}$).

In Vitro Cytotoxicity.—A549, a human non-small cell lung carcinoma cell line, was used to perform three-day cytotoxicity studies. A549 cells were cultured in RPMI-1640 medium

containing 10% FBS. The cells were plated in 96-well plates, 5,000 cells/well, and incubated at 37 °C for 24 h. After incubation, the cells were treated with 10 μ L of each solution: emulsion solutions containing paclitaxel (0.04, 0.4, 4, 400, 4,000, 40,000 nM in relation to paclitaxel concentration), standard paclitaxel solutions (0.04, 0.4, 4, 40, 400, 4,000 nM in relation to paclitaxel concentration), and emulsion solutions with no paclitaxel (17 nM, 1,700 nM, 17 μ M in relation to polymer concentration) diluted in 90 μ L of fresh medium and allowed to incubate for 24 h at 37 °C. Paclitaxel standard concentrations were prepared at least three magnitudes above and two below its reported IC₅₀ for the A549 cell line, 4 nM.³⁴ On the third day, all liquid was removed from each well and 100 μ L of CellTiter-Blue reagent (CellTiter-Blue cell viability assay, Promega) was added to each well.³⁵ The cells were incubated for another 3 h at 37 °C. The fluorescence intensity at 560 nm was analyzed using a plate reader. The cell viability in each well was calculated relative to the untreated control wells, and each type of well was averaged ($n = 6$).

***In Vivo* Developmental Toxicity Study.**—Due to the novel nature of the M2F8H18 copolymer, there is no preceding toxicity data. As a result, a thorough study of developmental effects related to M2F8H18 was performed using an embryo–larval zebrafish model. This animal model was selected because zebrafish eggs remain transparent from fertilization until the tissues become dense as a mature adult. Several developmental end points can be simultaneously monitored providing valuable developmental toxicity data for a variety of chemicals.³⁶ Following short-term exposure, survival, and hatching, non-lethal malformations were monitored including curved body axis and pericardial edemas. Mortality rate was also monitored.

Zebrafish (*Danio rerio*) of the AB strain were obtained from Dr. Michael Taylor at the University of Wisconsin—Madison School of Pharmacy, where the fish were cultured until sexual maturation for crossing. The fish were maintained in a light/dark cycle of 14:10 h at 28.5 °C in egg water (0.03% Instant Ocean, Blacksburg, VA, USA). Zebrafish were fed live brine shrimp (*Artemia nauplii*) twice a day. Embryos were obtained from healthy adult fish with a ratio of 1:2 for female to male. Six breeding groups were placed in separate spawning aquariums, equipped with a mesh bottom to prevent the eggs from being cannibalized. Crossing was induced in the morning when the light was turned on. One hour later, eggs free of macroscopically discernible symptoms of infection and disease were collected, rinsed with egg water, and transferred into Petri dishes until chemical exposure.

The embryo–larval toxicity assay was carried out according to standard protocols. Briefly, 8 fertilized eggs of 2 h post fertilization (hpf) stage were placed into each well of a 24-well plate, and each was filled with 600 μ L of egg water. Six concentrations (1 mM, 333 μ M, 111 μ M, 37 μ M, 12.3 μ M, and 4.1 μ M) plus two controls were plated. The plate was covered and incubated at 28.5 °C in a light/dark cycle of 14:10 throughout the 96 hpf exposure period. The observations of zebrafish development were made directly in the well using a stereomicroscope (Nikon SMZ18) every 24 h. End points including mortality, spontaneous movement, hatching success, pericardial edema, and curved body axis were selected to monitor the effects of M2F8H18. Embryos and larvae were considered dead when no heartbeat was observed. The number of hatched embryos and a cumulative mortality tally were recorded every 24 h from 2 hpf. The number of larvae displaying pericardial edemas or

curved body axes was also recorded every 24 h from 2 hpf. At 96 hpf, following the final observations, representative larvae were anesthetized with 0.4% tricaine mesylate solution and mounted on Petri dishes using low melting point agarose. The larvae were then photographed using a high-definition color microscope camera (Nikon DSFi2) and, finally, euthanized.

Magnetic Resonance Imaging (MRI).—Samples were prepared fully concentrated, without any dilution. The nuclear magnetic resonance (NMR) internal temperature was maintained at 25 °C. The ^{19}F relaxation parameters T_1 and T_2 , of the perfluoro-15-crown-5-ether present in the nanoemulsions, were measured on a Varian Unity-Inova 500 MHz (11.7 T) NMR spectrometer. The T_1 was determined using an inversion recovery experiment acquired with 12 independent, quadratically spaced variable (τ) values covering a range up to 10 times the estimated T_1 , 0.75 s. The T_2 was determined using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence experiment acquired with 12 independent, quadratically spaced variable (τ) values covering a range up to 10 times the estimated T_2 , 0.32 s. For T_1 and T_2 measurements: 90° pulse = 17.1 μs , nt = 16, spectral window (T_1) = 46948.4 Hz, and spectral window (T_2) = 46948.4 Hz.

MR images were acquired using an Agilent 4.7 T small animal horizontal bore scanner using a home-built ^{19}F quadrature volume coil with a 1.5 in. diameter and a 3 in. length. The temperature was maintained at 25 °C. A nanoemulsion stock of M2F8H18 was prepared by direct dilution of the nanoemulsion to the highest phantom concentration in sterile, normal saline (0.9% (w/w) sodium chloride). Lower phantom concentrations were made as serial dilutions from the stock solution in sterile, normal saline (0.9% (w/w) sodium chloride).

Nanoemulsion formation was confirmed by DLS. Phantom nanoemulsion solutions at PFCE concentrations of 154 mM, 61 mM, 31 mM, 3 mM, and 0 mM were transferred to polystyrene microcentrifuge tubes. ^1H images of the phantoms were acquired using a gradient echo pulse sequence with $0.19 \times 0.19 \times 2 \text{ mm}^3$ spatial resolution, $48 \times 48 \text{ mm}^2$ field of view (FOV), 8.82 ms TR, 4.43 ms TE, 20° flip angle, 195.3 Hz/voxel, 16 averages, and 36.2 s imaging time. ^{19}F images of the phantoms were acquired using a fast spin echo pulse sequence with a $0.25 \times 0.25 \times 2.0 \text{ mm}^3$ spatial resolution, $48 \times 48 \text{ mm}^2$ FOV, 0.500 s TR, 16.08 ms TE, echo train length of 8 echoes, 104.2 Hz/voxel, 50 averages, and 10 min 1 s imaging time. Image background threshold was adjusted to only view above 5% voxels.

Synthesis.

“BnOF8OH” 1H,1H,10H,10H-Monobenzyloxy-perfluorodecan 1-ol (1).: To a dry round-bottom flask were added 1H,1H,10H,10H-perfluorodecane-1,10-diol (8.5 mmol, 4 g) and anhydrous dimethylformamide (40 mL). This solution was stirred under argon for 10 min followed by the addition of sodium hydride (8.5 mmol, 0.20 g). This mixture was sonicated, under argon, at room temperature for an additional 10 min, and then benzyl bromide (8.5 mmol, 1 mL) was added dropwise. The reaction was sonicated for 3 h under argon. The reaction mixture was then neutralized with 5% HCl and extracted with ethyl acetate, saturated aqueous NaHCO_3 solution, and brine. The organics were dried with MgSO_4 , concentrated *in vacuo*, and purified via silica gel column chromatography. A gradient of 0–

15% ethyl acetate–hexanes was used. The separation was monitored with thin layer chromatography (TLC) and KMnO₄ staining. Isolated product resulted in 1.77 g (37.5% yield). ¹H NMR (500 MHz, CDCl₃): δ 7.35 (m, 5H), 4.68 (s, 2H), 4.10 (td, *J* = 15.2, 13.0, 7.6 Hz, 2H), 3.94 (t, *J* = 13.8 Hz, 2H), 1.95 (t, *J* = 7.5 Hz, 1H). ¹⁹F NMR (470 MHz, CDCl₃): δ –119.36 (t, *J* = 13.7 Hz), –121.92 (m), –122.42 (t, *J* = 13.6 Hz), –123.31 (bs), –123.57 (bs).

“H18OMs” Octadecyl Methanesulfonate (2): To a dry round-bottom flask were added anhydrous dichloromethane (100 mL), 1-octadecanol (21 mmol, 5.6808 g), and triethylamine (51 mmol, 7.10 mL). This mixture was stirred and gently heated until all solid 1-octadecanol was dissolved. Then the flask was put into an ice bath under argon. This mixture was allowed to react for 30 min before methanesulfonyl chloride (27 mmol, 2.10 mL) was added dropwise. The solution turned slightly cloudy. After running overnight, the solution was diluted with more DCM and extracted with saturated aqueous NH₄Cl solution. The crude mixture was then dried with MgSO₄ and concentrated *in vacuo*. Isolated product resulted in 7.15 g (97.7% yield). ¹H NMR (400 MHz, CDCl₃): δ 4.22 (t, *J* = 6.6 Hz, 2H), 3.00 (s, 3H), 1.75 (p, *J* = 6.9 Hz, 2H), 1.39 (m, 3H) 1.26 (bs, 31H), 0.88 (t, *J* = 6.6 Hz, 3H).

“BnOF8OH18” 1H,1H,10H,10H-Monobenzyloxy-octadecyloxy-perfluorodecane (3): To a dry round-bottom flask were added anhydrous benzotrifluoride (50 mL) and **1** (3.4 mmol, 1.90 g). This mixture was flushed with argon before addition of sodium hydride (14 mmol, 0.3418 g). The mixture was stirred for 1 h before **2** was added (4.4 mmol, 1.5611 g). The mixture was then heated to reflux for 24 h. After running overnight, the solution was diluted with DCM and extracted with saturated aqueous NH₄Cl solution. The crude mixture was then dried with MgSO₄ and concentrated *in vacuo*. Purification was performed via silica gel chromatography using a gradient of 0–5% ethyl acetate–hexanes. The separation was monitored with thin layer chromatography and KMnO₄ staining. Isolated product resulted in 2.53 g (91.3% yield). ¹H NMR (500 MHz, CDCl₃): δ 7.35 (m, 5H), 4.68 (s, 2H), 3.93 (q, *J* = 13.6 Hz, 4H), 3.59 (t, *J* = 6.6 Hz, 2H), 1.60 (p, *J* = 6.7 Hz, 2H), 1.25 (m, 32H), 0.88 (t, *J* = 6.8 Hz, 3H). ¹⁹F NMR (470 MHz, CDCl₃): δ –119.75 (t, *J* = 15.7 Hz), –120.03 (t, *J* = 15.7 Hz), –121.96 (m), –123.40 (d, *J* = 78.8 Hz).

“HOF8OH18” 1H,1H,10H,10H-Octadecyloxy-perfluorodecan-1-ol (4).

Route 1: To a dry round-bottom flask were added reagent grade methanol (100 mL), anhydrous tetrahydrofuran (50 mL), and **3** (2.90 mmol, 2.34 g). This mixture was stirred and flushed with argon for 1 h, and then palladium on carbon catalyst (3.9 mmol, 0.4100 g) was added. This mixture was flushed with argon for one more hour, and then a balloon of hydrogen gas was dispelled into the flask with a bubbler attached. A second balloon of hydrogen gas was then put on the reaction. The reaction mixture was stirred, and the reaction was run for 24 h under static hydrogen atmosphere. To work up the reaction, the balloon was removed and the flask flushed with argon for 1 h again. The mixture was then filtered through Celite with copious rinsing with DCM. Solvent was removed *in vacuo*. Isolated product resulted in 2.25 g (96.2% recovered yield). ¹H NMR (500 MHz, CDCl₃): δ 4.10 (td, *J* = 14.0, 7.5 Hz, 2H), 3.92 (t, *J* = 14.0 Hz, 2H), 3.59 (t, *J* = 6.6 Hz, 2H), 1.93 (t, *J* = 7.6 Hz, 1H), 1.60 (p, *J* = 6.5 Hz, 2H), 1.25 (m, 24H), 0.88 (t, *J* = 6.9 Hz, 3H). ¹⁹F NMR (470

MHz, CDCl₃): δ -119.65 (m), -121.96 (m), -122.42 (t, J = 12.6 Hz), -123.51 (d, J = 51.2 Hz).

Route 2.: To a dry round-bottom flask were added 1H,1H,10H,10H-perfluorodecane-1,10-diol (14.4 mmol, 6.6557 g) and anhydrous dimethylformamide (220 mL). The mixture was stirred and flushed with argon until fully dissolved, and then sodium hydride (14.5 mmol, 0.3482 g) was added. This was sonicated for 30 min, under argon, before **2** (14.3 mmol, 5.0 g) was added. The reaction mixture was sonicated for 21 h under argon. The reaction mixture was then neutralized with 5% HCl and extracted with ethyl acetate, saturated aqueous NaHCO₃ solution, and brine. The crude mixture was dried with MgSO₄ and concentrated *in vacuo*. Purification was performed using automated column chromatography, CombiFlash, using a silica gel column. A gradient of 0–20% ethyl acetate–hexanes was used. The separation was monitored using an evaporative light scattering detector (ELSD). Isolated product resulted in 1.84 g (17.9% yield). ¹H NMR (500 MHz, CDCl₃): δ 4.10 (td, J = 15.5, 13.1, 7.6 Hz, 2H), 3.92 (t, J = 13.9 Hz, 2H), 3.59 (t, J = 6.6 Hz, 2H), 1.96 (t, J = 7.5 Hz, 1H), 1.60 (p, J = 7.5 Hz, 2H), 1.25 (m, 33H), 0.88 (t, J = 6.9 Hz, 3H). ¹⁹F NMR (470 MHz, CDCl₃): δ -119.63 (t, J = 13.7 Hz), -121.95 (m), -122.43 (t, J = 13.5 Hz), -123.53 (d, J = 46.5 Hz).

“M2OMs” Monomethyl Poly(ethylene glycol) Methanesulfonate (5).: To a dry round-bottom flask, in an ice bath and under argon, were added anhydrous dichloromethane (50 mL), poly(ethylene glycol) monomethyl ether 2,000 (2.5 mmol, 5.0195 g), and triethylamine (7.5 mmol, 1.50 mL). This was allowed to react for 30 min before methanesulfonyl chloride (6.25 mmol, 0.50 mL) was added dropwise. After running overnight, the reaction mixture was diluted with more DCM and extracted with saturated aqueous NH₄Cl solution. The crude mixture was dried with MgSO₄ and concentrated *in vacuo*. Purification was performed on the crude mixture dissolved in minimal DCM via precipitation of pure **5** with cold ether in a dry ice/acetone bath. Isolated product resulted in 4.93 g (94.1% yield). ¹H NMR (400 MHz, CDCl₃): δ 4.38 (m, 2H), 3.64 (m, 182H), 3.38 (s, 3H), 3.09 (s, 3H).

M2F8H18 (6).: To a dry round-bottom flask were added **4** (3.7 mmol, 2.63 g) and anhydrous benzotrifluoride (150 mL). This mixture was cooled over ice and flushed with argon, and then sodium hydride was added (7.4 mmol, 0.1831 g). This reaction mixture was stirred for 30 min, and then **5** was added (1.9 mmol, 3.4526 g). The flask was heated to reflux and the mixture stirred at reflux for 5 days. Upon completion, the reaction mixture was cooled, diluted with DCM, and extracted with saturated aqueous NH₄Cl solution. The crude mixture was dried with MgSO₄ and concentrated *in vacuo*. Purification was performed via automated column chromatography, CombiFlash, using a C18 column and reverse phase conditions of a 90–0% water–methanol (0.1% formic acid) to 0–100% dichloromethane–methanol gradient. Polymer purity was confirmed via NMR, HPLC, and MALDI-MS. Isolated product resulted in 2.57 g (83.9% yield). ¹H NMR (400 MHz, CDCl₃): δ 4.04 (t, J = 14.1 Hz, 2H), 3.92 (t, J = 14.0 Hz, 2H), 3.79 (m, 3H), 3.64 (m, 174 H), 3.47 (t, J = 5.0 Hz, 1H), 3.38 (s, 3H), 60 (p, J = 7.0 Hz, 3H), 1.26 (m, 36H), 0.88 (t, J = 6.7 Hz, 4H). ¹⁹F NMR (376 MHz, CDCl₃): δ -119.73 (dt, J = 58.9, 11.9 Hz), -121.97 (m), -123.47 (bs). MALDI-

MS $[M + Na]^+$ calculated for $C_{113}H_{212}F_{16}O_{44}Na^+$: 2600.40 m/z ; found, 2600.584 m/z . $[M + K]^+$ calculated for $C_{113}H_{212}F_{16}O_{44}K^+$: 2616.37 m/z ; found, 2616.556 m/z .

RESULTS AND DISCUSSION

Synthesis of M2F8H18 Semifluorinated Polymer.

The synthesis of M2F8H18 was performed using two different routes adapted from previous work in the Mecozzi group^{37,38} as well as other published works.³⁹ The nomenclature for the polymer is based on the three different blocks: M x represents methoxy capped poly(ethylene glycol) where x is the average molecular weight in the thousands, F y represents the fluorocarbon block where y is the number of carbon atoms substituted with fluorine atoms, and H z represents the hydrocarbon block where z is the number of carbon atoms substituted with hydrogens. Route 1 (see Scheme 1) begins with the monobenzylation of 1H,1H,10H,10H-perfluorodecane-1,10-diol under basic conditions and sonication to yield the protected fluororous alcohol, **1**. Next, 1-octadecanol was mesylated under basic conditions to isolate **2**. Compounds **1** and **2** were coupled through a reflux reaction under basic conditions to yield the benzylated diblock intermediate, **3**. Hydrogenation of **3** catalyzed by palladium on carbon resulted in the alcohol diblock intermediate, **4**. Then, methoxy capped poly(ethylene glycol) (mPEG) with average molecular weight of 2,000 was mesylated under basic conditions, **5**. Finally, the alcohol diblock intermediate **4** was coupled to mesylated mPEG **5** through a reflux reaction under basic conditions to afford the final, desired M2F8H18 polymer, **6**. Compound **6** was purified using an automated CombiFlash system resulting in very high yields of the isolated polymer.

Alternatively, synthetic route 2 (Scheme 2) begins with the mesylation of 1-octadecanol under basic conditions to produce compound **2**. Next, 1H,1H,10H,10H-perfluorodecane-1,10-diol was coupled directly to **2** without prior protection steps (Scheme 1). This coupling reaction occurred under basic conditions and sonication to directly afford **4** after purification. The final two steps mimic what was accomplished in Scheme 1 including mesylation of mPEG **5**, and the final coupling of **4** and **5** under basic conditions and reflux. The final M2F8H18 polymer **6** was once again purified using an automated CombiFlash system.

Synthetic route 1 proved to be reliable and high yielding, overall, though it does involve a more extensive set of reactions including a protection and deprotection sequence. On the other hand, synthetic route 2 significantly shortened the overall reaction scheme by two steps but with a moderate loss of yield in the initial reactions.

Physicochemical Characterization of Polymer Aggregates.

The CMC of the M2F8H18 micellar aggregates was measured using surface tensiometry. The concentration that induces aggregation for M2F8H18, 2.2 μM , is an intermediate value compared to the related polymers M1F13 and M2H18 (Figure 2). A specific focus was placed on M2H18 due to its similarity in structure to the new M2F8H18 polymer, minus the fluorocarbon block. The standard polymer, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000], M2DSPE, was also

measured for reference and found to aggregate at a concentration of 12.6 μ M. The size of the micellar aggregates was also analyzed using DLS (Table 1). Based on aggregate size (Table 1) it can be concluded that M2F8H18 forms compact, stable particles.

Nanoemulsion Preparation and Stability.

Formulation of M2F8H18 nanoemulsions with MCT or an MCT/PTX combination was very successful, resulting in opaque, milky colloidal solutions with particle sizes centered around 230 nm initially. High concentrations of paclitaxel, from 1 to 4 mg of drug per mL of nanoemulsion, were loaded into the core of the particles. Nanoemulsions containing the highest concentrations of paclitaxel were stable for over one year, showing no signs of phase separation. The M2F8H18-based nanoemulsions also exhibited very minimal Ostwald ripening (Figure 3), one of the most common mechanisms leading to the growth and destabilization of emulsion particles, resulting in consistent nanoparticle size over time. The nanoemulsion sizes observed for M2F8H18 are appropriate to take advantage of the EPR effect and significantly below the FDA standard size cutoff of 500 nm for intravenous delivery of nanoparticles.^{40,41} The addition of the PFCE imaging agent resulted in increased colloidal stability and better drug retention, as hypothesized by the design of the M2F8H18 copolymer. This added stability from the fluororous excipient further supports the idea that the more dense the fluororous shell surrounding the oil droplet, the slower the drug release will be, which is critical for reducing systemic toxicity during the administration of chemotherapeutics.

In Vitro Drug Release Profile.

The *in vitro* paclitaxel release profile for M2F8H18 showed slow, steady release with highly sustained half-lives. Burst release is a common issue exhibited by many nanoparticles, but no trace of that is seen here. The linear M2F8H18 nanoemulsion without the imaging agent showed a promising half-life ($t_{1/2}$) of 69.5 h (Figure 4). This is an improvement compared to other drug delivery nanocarriers. For instance, reported *in vitro* paclitaxel release half-lives for micellar delivery systems range from 2 to 25 h.^{42–46} The observed controlled release of the M2F8H18 nanoemulsions can be attributed both to the penetration of the long hydrocarbon chains of M2F8H18 into the MCT oil droplet, where the drug is solubilized, and to the perfluorocarbon shell surrounding the oil droplet. The fluororous shell provides a barrier to modulate drug release from the internal oil core while at the same time providing enhanced stability to the nanoparticles. The effect of the fluororous shell can be appreciated by comparing drug release half-lives from fluororous M2F8H18-based nanoemulsions to the M2H18-based nanoemulsions devoid of a fluororous block: 69.5 and 52.9 h, respectively. Upon addition of the PFCE imaging agent to the M2F8H18 nanoemulsions, up to a 23.5% v/v, the drug release is even further modulated ($t_{1/2}$ of 92.4 h, Figure 4). Thus, not only does the PFCE add imaging potential to the nanoparticles but it also affords enhanced modulation of drug release.

In Vitro and *in Vivo* Toxicity Profile.

Cellular cytotoxicity was examined *in vitro* using A549 non-small cell lung carcinoma cells. The A549 cells were selected due to their robust nature and frequent use in the field. The cells were treated with three solutions including a M2F8H18 nanoemulsion containing 4

mg/mL paclitaxel with 4 mL of MCT, a M2F8H18 nanoemulsion containing no paclitaxel with 4 mL of MCT, and paclitaxel standard solutions prepared with low concentrations of dimethyl sulfoxide (DMSO). Because DMSO has been shown to be toxic to A549 cells at high concentrations, DMSO cytotoxicity was also monitored in this study.⁴⁷ Paclitaxel standard concentrations were centered around the reported IC₅₀ for the A549 cell line, 4 nM.³⁴ As seen in Figure 5, the wells containing 0.05% DMSO, which is the highest concentration used in this study, showed little to no cell inhibition, confirming that it was appropriate to use low concentrations of DMSO in solution preparation without any cytotoxic effects. The wells treated with the M2F8H18 nanoemulsion devoid of drug exhibited very high cell viability, confirming that both the polymer and MCT oil do not inhibit cell growth. The M2F8H18 nanoemulsion loaded with paclitaxel did inhibit cell growth at similar concentrations as the standard paclitaxel solutions. In fact, the paclitaxel delivered via the nanoemulsion inhibited cell growth at an even lower concentration compared to the standards. This data suggests that the chemotherapeutic containing nanoemulsions can successfully inhibit cancer cell growth, as they were designed to do.

The M2F8H18 polymer was also screened for potential intrinsic toxicity due to the novel nature of the polymer using a zebrafish model, the primary model used to study nanotoxicity.^{48–50} A thorough study of developmental effects related to M2F8H18 was performed using an embryo–larval zebrafish model. This animal model was selected because zebrafish eggs remain transparent from fertilization until the tissues become dense as a mature adult. Several developmental end points can be simultaneously monitored providing valuable developmental toxicity data for new chemicals. After crossing of the fish and fertilization of the eggs had occurred, the embryos were collected and plated. The embryos were then treated with M2F8H18 and positive control solutions of the following concentrations: 1 mM, 333 μ M, 111 μ M, 37 μ M, 12.3 μ M, and 4.1 μ M. The embryos were then incubated, monitored, and photographed over a 96 h experiment period. Survival rates for the nontreated control fish (Figure 6) were very high, as would be expected from healthy zebrafish at 96 hpf. The positive control for the experiment, perfluorooctanoic acid (PFOA), was selected for its documented toxicity to zebrafish and the contrasting difference between toxic perfluoro acids and benign perfluorocarbon-containing copolymers.^{51,52} The PFOA caused mortality in all fish at the highest concentration of 1 mM, resulting in 0% survival at 96 hpf. At PFOA concentrations of 333 μ M, 67% of fish survived and some developmental malformations were observed including curved body axis and mild pericardial edemas. The PFOA results are varied at concentrations at and below 111 μ M, indicating that sublethal concentrations may have been reached. No other malformations were noted at the lower PFOA concentrations. In great contrast to PFOA, the novel M2F8H18 polymer induced 100% survival in the fish of this study at the highest concentration of 1 mM. The small decrease in survival rate, 78%, seen at 111 μ M is thought to be a random anomaly based on the collective zebrafish data. None of the fish exposed to M2F8H18 in this study exhibited malformations such as curved body axis or pericardial edemas, as well. This data suggests that there are no developmental toxicity concerns involving 1 mM and below concentrations of the newly synthesized M2F8H18 polymer. Research in this area may progress to further *in vivo* stages.

¹⁹F-MR Imaging Characterization.

M2F8H18-based nanoemulsions can be loaded with high concentrations of PFCE. The addition of this fluororous imaging agent enhances the drug release profile of the nanoemulsions, slows drug release, and provides further stability to the colloidal particles. Next, the imaging potential of this molecule was investigated. Preliminary ¹⁹F NMR studies of PFCE at 11.7 T show an intense, single peak at δ –91.8 ppm with CFC1₃ as an internal reference.²³ This pattern is due to the 20 magnetically equivalent fluorine atoms and ring flexibility in solution of the PFCE. The ¹⁹F relaxation parameters T_1 , longitudinal relaxation or delay between pulses, and T_2 , transverse relaxation or signal decay over time, were also measured to monitor potential PFCE use as an efficient contrast agent. The T_1 was determined using an inversion recovery experiment while T_2 was determined using a Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence experiment. The resulting relaxation values show a relatively short T_1 value of 0.781 ± 0.002 s allowing for quick recovery between pulses and increased scanning efficiency. Additionally, the T_2 relaxation was a relatively long value of 0.163 ± 0.006 s that should avoid most signal decay over time. Based on the single resonance intensity, high payload concentration within the nanoemulsions, and good relaxation values, PFCE shows great promise to be used as a ¹⁹F-MRI contrast agent through a fluororous nanoemulsion. Following preliminary work to establish the relaxation parameters of PFCE, ¹⁹F-MRI phantom images of the PFCE loaded nanoemulsions were acquired using a 4.7 T small animal MRI instrument. The ¹H images (Figure 7a) were acquired over an imaging time of 36.2 s, while the ¹⁹F images (Figure 7b) were acquired over an imaging time of 601 s. For the ¹H images, the instrument was tuned to the highest intensity proton resonance within the nanoemulsion which equated to the saline peak. In a similar manner, for the ¹⁹F images, the instrument was tuned to the highest intensity fluorine resonance within the nanoemulsion which equated to the PFCE imaging agent peak. ¹⁹F-MRI signal intensity was extremely high with the fully concentrated nanoemulsion sample (top center, Figure 7) with PFCE concentration of 154 mM. Excellent signal intensity can also be seen at the 61 and 31 mM concentration with the visual limit of detection falling above 3 mM here. Calculations to determine the estimated minimum limit of detection, based on the minimum signal-to-noise ratio (SNR) and linear fit of PFCE concentration to ¹⁹F-MRI signal intensity, predict that concentrations as low as 1.3 mM PFCE can be resolved. These are low concentrations easily achievable with the described nanoemulsions and with applicable translation to *in vivo* work, as seen in the literature.^{26,53} If desired, image SNR can also be amplified further with a different pulse sequence or by using an instrument of higher field strength.⁵⁴ These data offer great potential for use of these theranostic nanoemulsions *in vivo*.

CONCLUSIONS

The novel, semifluorinated copolymer M2F8H18 was synthesized and thoroughly characterized. This redesigned ABC polymer forms highly stable, triphilic nanoemulsions due to the intrinsic driving force of the fluororous phase, with lifetimes up to 1 year. Very limited Ostwald ripening is observed for these particles, which remain well below the size of 400 nm for their full lifetime. The M2F8H18 surfactant can form therapeutic nanoemulsions where it stabilizes MCT oil droplets containing highly hydrophobic paclitaxel dissolved at

concentrations up to 4 mg/mL. No developmental toxicity was found for this polymer following *in vivo* studies in zebrafish. M2F8H18 nanoemulsions with a paclitaxel payload inhibit cancer cell growth in a concentration dependent manner. Drug release from the oil core of the particles is gradual and controlled due to the long, stabilizing hydrocarbon chains that penetrate the oil droplet and the intermediary fluorocarbon shell that acts as a time barrier to drug release. The addition of the fluorous imaging agent PFCE modulates drug release even further by sealing the fluorous shell of the polymer. Upon addition of the PFCE, these nanoemulsions can be formulated into theranostic systems with dual therapeutic and diagnostic character. High concentrations of the PFCE can be loaded into the nanoemulsions resulting in strong ^{19}F -MR signal due to its intense, single resonance composed of 20 magnetically equivalent fluorine atoms, as well as short T_1 and long T_2 . These studies show that the triphilic copolymer M2F8H18 can be used for the formulation of stable nanoemulsions that carry payloads of potent chemotherapeutic drug and ^{19}F -MRI contrast agent all wrapped into a powerful, theranostic system. Work is in progress for the translation of these M2F8H18-based therapeutic and theranostic nanoemulsions to *in vivo* models.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

ACN	acetonitrile
CMC	critical micelle concentration
DCM	dichloromethane
DLS	dynamic light scattering
DMSO	dimethyl sulfoxide
EPR	enhanced permeability and retention
MCT	medium chain triglycerides
MWCO	molecular weight cutoff
PTX	paclitaxel

PFCE	perfluoro-15-crown-5-ether
SNR	signal-to-noise ratio

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Figure 1.
Hypothesized organization of the ABC polymer M2F8H18 into therapeutic nanoemulsion particles.

Figure 2.
Chemical structure of M2F8H18 and related polymers.

Figure 3.

Change in particle size of M2F8H18 nanoemulsions over time as measured by DLS: (red) 4 mL of MCT only, (orange) 2 mL of MCT and 1 mg/mL paclitaxel, (blue) 4 mL of MCT and 4 mg/mL paclitaxel, (green) 2 mL of MCT and 10% v/v PFCE, (purple) 2 mL of MCT, 1 mg/mL paclitaxel, and 23.5% v/v PFCE. All nanoemulsions were prepared in 0.9% w/w NaCl. Note: Error bars are removed here for clarity. They are available in the Supporting Information; see Figure S2 and Table S1.

Figure 4.

In vitro nanoemulsion drug release profiles monitored for 168 h: (blue) M2F8H18 with MCT only, (green) M2F8H18 with MCT and PFCE.

Figure 5. A549 cell viability studies: (blue) nontreated control, (orange) 0.05% DMSO control, (purple) M2F8H18 nanoemulsion with MCT but no drug, (green) M2F8H18 nanoemulsion loaded with 4 mg/mL paclitaxel and MCT, (red) paclitaxel standard solutions in DMSO.

Figure 6. Zebrafish survival rates after exposure for 96 hpf: (blue) nontreated control, (red) positive control of perfluorooctanoic acid (PFOA), (green) M2F8H18 polymer.

Figure 7.

(a) ^1H -MR and (b) ^{19}F -MR phantom images taken at 4.7 T. Image background threshold was adjusted to only view above 5% voxels. Scale bar indicates signal intensity based upon signal-to-noise ratio (SNR). From top center moving clockwise, the concentration of PFCE in the M2F8H18 nanoemulsion is 154 mM, 61 mM, 31 mM, 3 mM, and 0 mM. Strong signal intensity can be viewed at 31 mM and above.

Scheme 1.
Synthesis of M2F8H18 Copolymer

Scheme 2.

Alternative, Shortened Synthesis of M2F8H18 Copolymer

Table 1.

Physicochemical Characteristics of Aggregates

polymer	CMC log (M)	micellar particle size (nm)
M2F8H18	-5.7 ± 0.2	16.3 ± 4.5
M1F13	-6.1 ± 0.1	17.2 ± 1.9
M2H18	-5.1 ± 0.1	12.2 ± 3.1
M2DSPE	-4.9 ± 0.2	13.9 ± 1.6