Preparation of Perfluorocarbon/Fluoroalkyl Polymer Nanodroplets for Cancer-targeted Ultrasound Contrast Agents

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Perfluorocarbon (PFC) nanodroplets formed from PEG-*b*-poly(fluoroheptyl aspartate) were developed for a cancer-targeted ultrasound (US) contrast agent. 10% esterifed fluoroalkyl polymer (F10) was the optimal composition for PFC solubilization in aqueous solution. PFC/F10 nanodroplets showed echogenicity with US irradiation, and long-term stability of PFC nanodroplets at 4 °C was confirmed.

Ultrasound irradiation is an important technology for diagnostic imaging like MRI and X-ray imaging and is also useful for therapeutic purposes. Ultrasound has a number of attractive advantages; noninvasiveness, portability, real time imaging, and relatively low cost. Thus, effective ultrasound (US) contrast agents[1,2] and US-mediated therapeutic agents[3–6] have been actively investigated. Particularly, microbubbles have been successful US contrast agent and US-mediated therapeutic agents. From the physical characteristics, microbubbles mainly provide images of vascularity,[7] and most microbubbles show no tumor-targeting properties by extravasating through defective vasculature and by accumulating nanoparticles at tumor sites (EPR effect). Therefore, nanosized US contrast agents have been investigated.

Maruyama's group has developed a bubble liposome which consists of liposomes encapsulating perfluoropropane as an US contrast gas.[8] Rapoport's group has studied perfluoropentane (PFP) nanodroplets formed with amphiphilic block copolymers.[9] Encapsulated PFP nanodroplets do not show US contrast. Above physiological temperature, encapsulated PFP nanodroplets form microbubbles easily through vaporization (bp PFP is 29 °C), exhibiting echogenicity. Rapoport et al. suggested that nanodroplets could extravasate at tumor sites and coalesce into larger and highly echogenic microbubbles, providing strong tumor contrast in ultrasonography.

As shown in Rapoport's work, polymer nanocarrier-encapsulated PFP should be a promising US contrast agent. However, it is difficult to retain nanosize of the PFP nanodroplets because of easy nanodroplet/microbubble conversion from the low PFP boiling point. To solve this problem, we applied amphiphilic fluorinated polymers, PEG-*b*-poly(fluoroheptyl aspartate)s, as PFP carriers. The fluorinated polymers have high compatibility with perfluorocarbons, and the block copolymer can form a hydrophobic core/hydrophilic shell structure easily. In this paper, we measured PFP encapsulation capacity of the amphiphilic fluorinated polymers and optimized composition as a nanosized polymeric carrier for PFP encapsulation. Moreover, we also investigated the echogenicity of the resulting nanodroplets with US irradiation.

PEG-*b*-poly(fluoroheptyl aspartate)s were prepared according to a modified procedure.[10] We used 4,4,5,5,6,6,7,7,7-nonafluoroheptyl iodide ($C_7H_6F_9I$) as a fluorinated hydrophobic unit.

Table 1. Concentration of encapsulated PFP and weight average diameters of nanodroplets at 10 °C

	Esterification/%	PFP in aq /mg·mL^{-1}	Diameter/nm
F0	0	0	—
F5	5.9	46.1	609.0 ± 140.2
F10	13.5	108.9	693.9 ± 200.9
F20	22.3	31.4	568.2 ± 129.7
F40	38.5	2.7	562.5 ± 108.1
F70	67.0	1.9	361.7 ± 76.4
H10	13.2	46.0	269.0 ± 68.1
F10(C9)	13.5	45.5	332.4 ± 110.8
F10(C11)	13.6	22.4	657.5 ± 143.9

PFP was mixed with perfluorohexane (PFH), which has an elevated boiling point (59 °C), in order to form stable perfluorocarbon nanodroplets and suppress echogenic error in the nanodroplets. The ratio of PFP and PFH was 85:15 (vol). The PFC mixture was encapsulated into nanodroplets by vigorous stirring in aqueous polymer solutions below 10 °C (described in Supporting Information[11]). PFP concentrations in the PFC/polymer mixture were measured by gas chromatography (GC) and summarized in Table 1.

First, we compared PFC encapsulation capability between the fluorinated polymer and a nonfluorinated polymer, H10. H10 was a nonfluorinated heptyl ester. The PFP concentration in H10 was about 40% lower than that of F10. This difference clearly indicated that PFP solublization in fluoroalkyl ester is greater than in nonfluorinated ester.

We examined effects of esterification degree on PFC encapsulation. First, we expected that highly esterified fluoroheptyl polymer would have higher compatibility with PFC than low esterified materials, thus F70 should have the highest encapsulation capability for PFC. However, F10 showed the highest PFP concentration in polymer solution. Moreover, PFP concentration in a lower esterified fluoroheptyl polymer solution, F5, was lower than that of F10. These results indicated that around 10% esterification was optimal for PFP encapsulation with the fluoroheptyl ester.

We hypothesized a mechanism as described below (Figure 1). Fluoroalkyl ester groups are highly compatible with PFC; therefore, the fluoroheptyl polymers would orient the fluoroheptyl pendant group into the PFC droplets. The number of fluoroheptyl groups oriented into one PFC droplet would be defined because the surface area of the PFC droplets would be constant. In the case of F10, a large quantity of F10 should be used for PFC solubilization because F10 has a small number of fluoroheptyl groups in the polymer. Therefore, PFC droplets formed from F10 polymer would expose many PEG units to aqueous media and show a high PEG density on the surface of the PFC

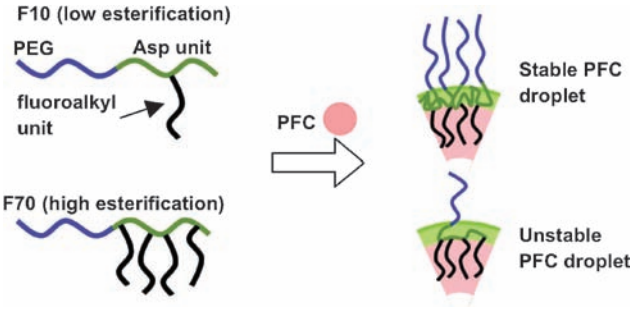

Figure 1. Mechanism of PFC encapsulation with fluoroalkyl polymers.

droplet. On the other hand, high-esterified polymer such as F70 would expose lower numbers of PEG units than F10 because many fluoroheptyl units are present in the PFC droplets from one polymer. Therefore, the PFC droplets formed with F70 would have a low PEG density on the surface of the droplet. The high density of PEG units should stabilize the PFC droplet compared with the low density case. Thus, F10 could solubilize the highest amount of PFP in the aqueous polymer solution. F5 would also show the high PEG density on the PFC droplet. However, the number of fluoroheptyl units in the polymer was too low to stabilize PFC droplets in aqueous media. Therefore, 10% esterification was the best composition for PFC encapsulation with the fluoroheptyl polymers.

Particle sizes of PFC droplets were measured with DLS at 10 °C. Most samples showed unimodal distributions (Figure 1Sa[11]), and the diameters were 300–700 nm at 10 °C (Table 1). These particle sizes were a bit larger than the optimal size for EPR effect (<200 nm). However, particle size can be reduced with an extruder (Figure 1Sb[11]). Moreover, the size of PFC droplets was observed to change with temperature. The particle size of the PFC/F10 droplets at 37 °C was 186.9 ± 42.5 nm (Figure 2a). The mechanism of droplet size change with temperature was not clear. However, the diameter of the PFC droplets formed with the fluorinated polymers can be tuned.

Effects of chain length of the fluoroalkyl unit on PFC encapsulation were examined. F10(C9) and F10(C11) were a fluorononyl and a fluoroundecyl unit, respectively. PFP concentrations of these samples showed lower values than that of F10, and the PFP concentration decreased with the increase of chain length of the fluoroalkyl unit. High hydrophobicity might be stabilized large PFC droplets which precipitate easily due to large amount of PFC. This result implied balance of hydrophobicity and the ratio of esterification of the fluorinated polymers should be a very important factor for the PFC encapsulation.

Finally, we confirmed echogenicity of PFC droplets prepared from F10 with US irradiation (Figure 2b). A PFC droplet embedded into a polyacrylamide gel did not show any contrast before US irradiation because the PFC inside the droplet was liquid. However, the PFC droplets showed high contrast in an US image after the US irradiation because PFC became gas, which has high echogenisity. This meant that the PFC (liquid

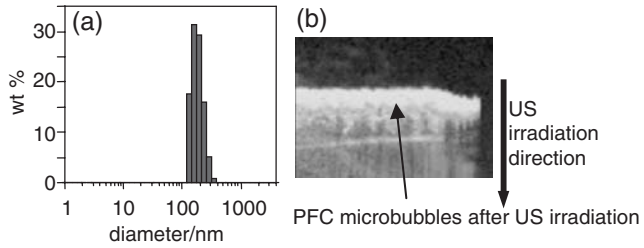

Figure 2. Size distribution of a PFC/F10 droplet at 37 °C (a) and US image of the droplet after US irradiation (b).

phase) droplet became the PFC (gas phase) microbubble by means of US irradiation and had sufficient capability as an US contrast reagent. Moreover, loss of PFP from PFC droplets at 4 °C was little even after 4 weeks (Figure 2S[11]). From this result, long-term stability of PFC droplets at 4 °C was confirmed.

In this paper, we reported that PFC droplets formed from PEG-*b*-poly(fluoroheptyl aspartate) were developed as an US contrast reagent. We confirmed that a fluoroheptyl unit was the optimal hydrophobic group for PFC solubilization to an aqueous solution, and 10% esterified polymer was the best composition for the preparation of the PFC droplet. The reported PFC encapsulation method is very easy to manipulate compared with other preparation methods, thus the reported method would be a promising procedure for preparations of US contrast agents. In future study, we will evaluate tumor accumulation of the PFC droplets and echogenicity in mice.

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11 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

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