Novel ^{19}F MRS/I Nanoprobe Based on pH-Responsive PEGylated Nanogel: pH-Dependent ^{19}F Magnetic Resonance Studies

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The pH-responsive PEGylated nanogels composed of the cross-linked poly[2-(*N*,*N*-diethylamino)ethyl methacrylate]-*co*-poly(2,2,2-trifluoroethyl methacrylate) gel core showed a remarkable on–off regulation of ^{19}F magnetic resonance signal intensity (T_2 values) as well as signal-to-noise ratios in response to extracellular pH 6.5 of tumor environment under ^{19}F magnetic resonance spectroscopic imaging (MRS/I), demonstrating the utility of the PEGylated nanogels as solid tumor-specific ^{19}F MRI/S nanoprobes.

^{19}F magnetic resonance spectroscopic imaging (^{19}F MRS/I) has been recognized as a powerful and noninvasive methodology for diagnosis of cancer, because there is no endogenous ^{19}F in the body that might be a source of background noise. Additionally, ^{19}F is 100% naturally abundant and has MR sensitivity nearly as high as that of protons.[1] Since long circulating nanosized (<100 nm) and PEGylated species[2] have been reported to accumulate in solid tumors through the enhanced permeability and retention (EPR) effect,[3] an important goal in tumor imaging is development of PEGylated ^{19}F nanoprobes. Furthermore, the extracellular pH of the tumor environment is usually 0.4 to 1.0 pH units lower than the physiological pH 7.4.[4] The ^{19}F MRS/I of tumors can be further improved by designing pH-responsive ^{19}F-nanoprobes that attenuate the ^{19}F MR signal outside of the tumor and switch on the signal inside the tumor.

In this regard, we recently first reported the preparation of pH-responsive and PEGylated ^{19}F nanoprobes based on PEGylated nanogels consisting of 1) a cross-linked poly[2-(*N*,*N*-diethylamino)ethyl methacrylate]-*co*-poly(2,2,2-trifluoroethyl methacrylate) (PEAMA-*co*-PTFEMA) gel core and 2) tethered PEG chains that bear an acetal group as a platform for installation of tumor-specific ligand molecules (Figure 1).[5] These pH-responsive nanoprobes showed remarkable activation of ^{19}F MR signals upon change of pH 7.4 to pH 6.5 even in the presence of 90% fetal bovine serum. The pH-dependent behavior of the PEGylated nanogel probes may result from the swelling (volume phase transition) of the gel core at acidic pH that leads to an increase in molecular motion of the ^{19}F atoms and longer T_2 (spin–spin) relaxation times. To validate this hypothesis the present study investigates the ^{19}F MR signals including T_1 (spin–lattice) and T_2 relaxation times and phantom of the nanogel probes and discusses the relevance of these characteristics to ^{19}F MRS/I of tumors. All together, based on these systematic measurements, we gain important insight into "on–off" regulation of the ^{19}F MR signals induced by the volume phase transition of the gel core in response to pH.

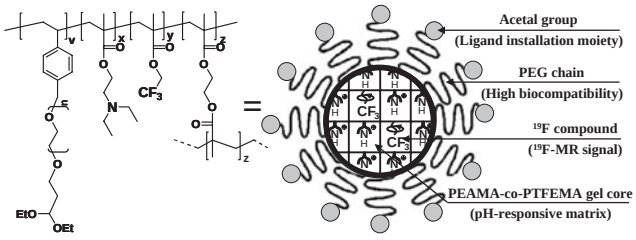

Figure 1. Schematic illustration of tumor-specific nanoprobe for ^{19}F MRS/I based on the pH-responsive PEGylated nanogels.

The pH-responsive PEGylated nanogel was prepared by emulsion copolymerization of EAMA (pH-responsive component), TFEMA (^{19}F compound), and PEG macromonomer bearing an acetal group at the α-end and a 4-vinylbenzyl group at the ω–end (acetal–PEG–Ph–CH=CH$_2$: M_n = 5030, M_w/M_n = 1.04) in the presence of ethylene glycol dimethacrylate (1.0 mol %) as a crosslinker. The emulsion copolymerization was carried out at a feed ratio of EAMA:TFEMA = 90:10 (mol %), since the highest ^{19}F MR signal intensity at extracellular pH 6.5 of the tumor environment was observed for the PEGylated nanogel containing PEAMA-*co*-PTFEMA gel core (90:10 mol %).[5] By decreasing the pH from 7.4 to 7.0, the

Table 1. Size, T_1 and T_2 relaxation times of ^{19}F for the pH-responsive PEGylated nanogels at pH 7.4, 6.5, and 5.5

pH	Diameter/nm[a]	T_1/ms	T_2/ms	S/N[b]
7.4	52	<30	<1	≈0
6.5	107	280	56.8	7.63
5.5	109	304	53.2	5.75

[a]Determined by DLS measurement. [b]Calculated from phantom images.

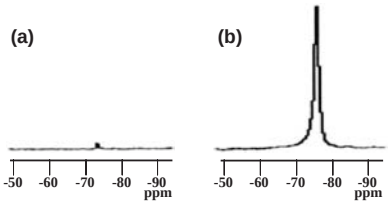

Figure 2. ^{19}F MR spectra of the pH-responsive PEGylated nanogels at (a) pH 7.4 and (b) pH 6.5.

diameter of the PEGylated nanogel increased proportionally with a unimodal size distribution ($\mu_2/\Gamma^2 < 0.19$), reaching 8.7-fold larger hydrodynamic volume at the extracellular pH 6.5 of tumor environments compared to that at the physiological pH 7.4, as shown in Table 1. This pH-induced size variation of the PEGylated nanogel was due to an increase in the ion osmotic pressure and solvation of the gel core caused by protonation of the amino groups in the PEAMA segment.[5,6] Figure 2 shows the ^{19}F MR spectra of the PEGylated nanogels ($[^{19}F] = 500\,\mu M$), as measured by ^{19}F MRS/I (7.0 T) at the physiological pH 7.4 and extracellular pH 6.5 of the tumor environment. T_1 and T_2 relaxation times of ^{19}F for the PEGylated nanogels at pH 7.4, 6.5, and endosomal/lysosomal pH 5.5[7] are also summarized in Table 1. Note that almost no narrow line width ^{19}F MR signals were observed at physiological pH 7.4 (Figure 2a). At this pH, the gel core collapsed to hydrophobic core as a result of the deprotonation of the amino groups. Furthermore, T_2 was found to be too short (T_2 estimated to be 100 μs based on signal linewidth), viz., the absence of ^{19}F MR signals is due to the broadening effect caused by the limited molecular motion of the ^{19}F compounds in the hydrophobic (solid-state) core.[8] In sharp contrast, the PEGylated nanogels (Figure 2b) showed clear ^{19}F MR signals at pH 6.5 along with a very broad peak not visible using normal imaging methods. The pronounced ^{19}F MR signals are due to the swelling as well as solvation of the gel core in response to acidic pH, leading to the increase in the molecular motion of the ^{19}F compounds. Indeed, T_2 values of the PEGylated nanogels at pH 6.5 and 5.5 were significantly longer than that at pH 7.4. Note that these facts are in accordance with the size variation of the PEGylated nanogels as function of pH. Thus, complete on–off regulation of T_2 values (^{19}F MR signal intensity) by the pH-responsive PEGylated nanogel are remarkable characteristics for the tumor-specific ^{19}F MRS/I nanoprobes.

Figure 3 shows the phantom images of both ^{19}F MRS/I for the PEGylated nanogels at pH 6.5 and ^{1}H MRI of the water as a reference obtained using the same volume coil transmit and surface coil receive system retuned to the ^{1}H frequency.[9] Since the signal at the top surface is stronger than that at the bottom for ^{19}F and ^{1}H phantom images arising from the reception profile of

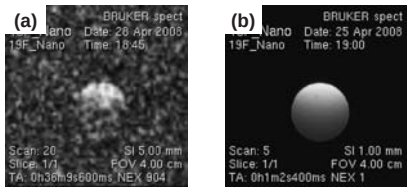

Figure 3. (a) ^{19}F MRI of the phantom containing the pH-responsive PEGylated nanogels at pH 6.5 ($[^{19}F] = 500\,\mu M$). (b) ^{1}H MRI of the phantom of water as a reference.

the surface coil, the signal-to-noise (S/N) ratios at the top surface of the images for the PEGylated nanogels at pH 7.4, 6.5, and 5.5 were measured to be ca. 0, 7.63, and 5.75, respectively. Significant increase in the S/N ratios in response to pH 6.5 and 5.5 is remarkable, which means that the PEGylated nanogels can be used for tumor-specific ^{19}F MRS/I nanoprobes.

In conclusion, the pH-responsive PEGylated nanogels showed a remarkable on–off regulation of T_2 values of ^{19}F as well as S/N ratio in response to the extracellular pH 6.5 of the tumor environment, indicating that the pH-responsive PEGylated nanogels are a promising approach to the creation of tumor-specific ^{19}F MRS/I nanoprobes to be used in vivo.

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