

Organic radical contrast agents based on polyacetylenes containing 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO): targeted MR/optical bimodal imaging of folate receptor expressing HeLa tumors in vitro and in vivo

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Purpose: As a metal-free magnetic resonance imaging (MRI) contrast agent, organic radicals have become a promising diagnostic tool for tumor detection.^{1,2} However, one major challenge of this vector is lack of specific delivery system for targeted cancer imaging. Here, we report the synthesis and characterization of folic acid (FA) conjugated multifunctional organic radical contrast agents (ORCsAs) for targeted bimodal MRI/optical imaging of tumors.

Methods: Polyacetylenes containing 2,2,6,6-tetramethyl-piperidinoxy and poly(ethylene glycol) were synthesized by metathesis polymerization of corresponding substituted acetylenes. The polyethylene glycol in polyacetylenes enabled covalent conjugation of carboxyl fluorescein and FA with hydroxyl groups to develop targeted multifunctional ORCAs (Fig.1).³ The targeted bimodal probe PA-TEMPO-FI-FA was fully characterized, and its binding specificity and subsequent internalization into FA receptor over-expressing Hela tumor cells was confirmed by fluorescence imaging and *in vitro* MRI. The nontargeted probe PA-TEMPO-FI was tested as the control group. The contrast enhancement in the Hela tumor-bearing mice was observed by MRI and optical imaging after intravenous injection of PA-TEMPO-FI-FA (2.5 mmol, 0.3 ml) through the tail vein. The T1-weighted spin-echo sequence (TR/TE 400/15ms, section thickness 3.0 mm)

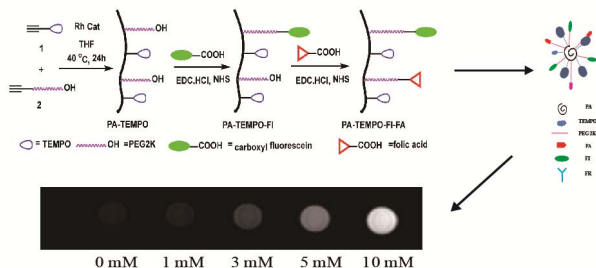


Figure 1. Synthesis of PA-TEMPO-FI-FA and T1-weighted MRI images of PA-TEMPO-FI-FA at various concentrations.

was acquired for MRI studies using a 3.0T clinical MRI scanner (Achieva TX, Philips Healthcare, Netherlands). After *in vivo* imaging, tumors were resected from the sacrificed mice for fluorescence immunohistochemistry.

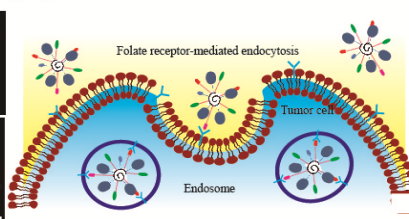
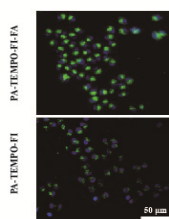
Results and discussions: Fluorescence microscopy and *in vitro* MRI (Fig.2) confirmed enhanced cellular internalization of PA-TEMPO-FI-FA through FA receptor-mediated delivery. Fig.3 shows sufficient tumor contrast observed by *in vivo* MRI and optical imaging. Fluorescence histological images demonstrated abundant accumulation of green fluorescence within the tumor, which was responsible for the signal increase.

Conclusion: We have developed fluorescent paramagnetic bimodal ORCAs for targeted MRI/optical imaging with high performance. The multifunctional ORCAs are expected to be widely exploited as cell-specific delivery vehicles for cancer imaging and therapy applications.

References: [1] Rajca A, et al, *J Am Chem Soc* 2012, 134, 15724. [2] Bye N, et al, *Langmuir* 2014, 30, 8898. [3] Liu R, et al, *J. Polym. Sci., Part A: Polym. Chem* 2008, 46, 4183.

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Fluorescence imaging



Cellular MRI

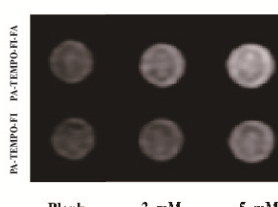


Figure 2. *In vitro* fluorescence imaging and MRI of HeLa cells incubated with PA-TEMPO-FI-FA and the control probe PA-TEMPO-FI. Schematic illustration of specific targeting of folate receptor on tumor cells by FA-modified ORCAs (middle).

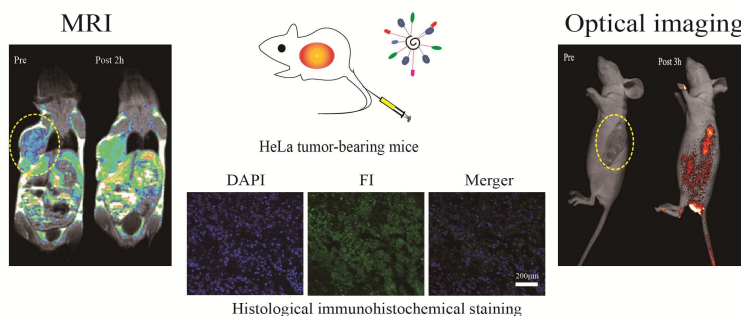


Figure 3. *In vivo* T1-weighted MRI color-maps and optical imaging of HeLa tumor-bearing mice and fluorescence immunostaining of the tumor section.