Self-assembled polymeric MR contrast agents for superfine microvasculature imaging

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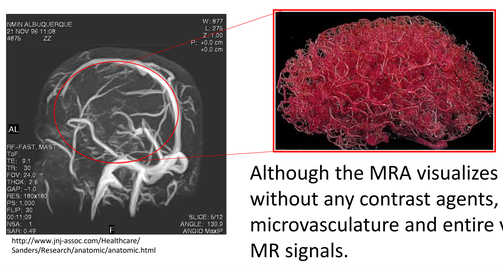
Introduction

Superfine microvasculature imaging on MRI scan by 8-arm PEG-FGd$_3$

Magnetic resonance angiography (MRA) is used clinically for diagnosis of the brain diseases. In our previous work, we succeeded in visualizing the superfine cerebrovasculature using Gd conjugated 8-arm polyethylene glycol bearing small amount of the fluorescein molecules as MR contrast agent[1]. Interestingly, the images were not acquired when Gd-conjugated 8-arm PEG without fluorescein conjugation was used.

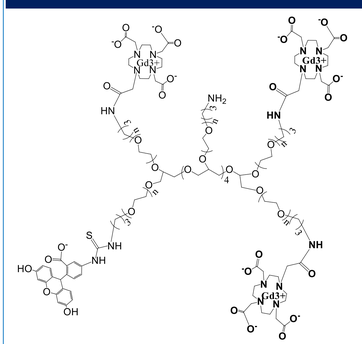
In this study, we **synthesized the 8-arm PEG having 15 kDa of molecular weight which was conjugated with one fluorescein and three Gd-chelate** (Figure 1). We investigate the self-assembled structure of the polymer at different concentrations by AFM and spectroscopic analysis. MR images of rat cerebrovasculature is also indicated and will be discussed its imaging resolution[2].

References:
1. Mahara A. et al., MacloBiosci, (2018) e1700391.
2. Mahara A. et al., Chem. Commun. (2020) 11807.



Although the MRA visualizes the main cerebral artery without any contrast agents, it is hard to visualize the microvasculature and entire vascular network due to low MR signals.

8-arm PEG-FGd$_3$



Molecular weight: 17kDa

Relaxivity rate

r_1: 10.5 × 10^{-3} M^{-1} s^{-1}

r_2: 13.5 × 10^{-3} M^{-1} s^{-1}

Figure 1. Chemical structure of 8-arm PEG-FGd$_3$

Results and Discussion

1. Hydrodynamic radius

Hydrodynamic radius of 8-arm PEG-FGd$_3$:
1400 nm at 200 mg/mL
40 nm at 10 mg/mL
8 nm at 1mg/mL

The radius was depended on the polymer concentration.

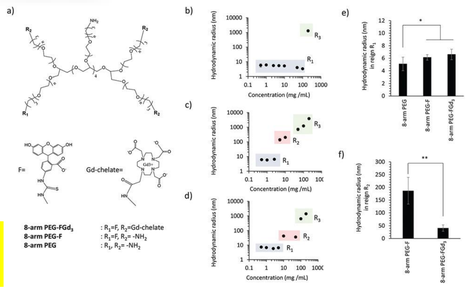


Figure 2. (a) Chemical structure and hydrodynamic radius of fluorescein and Gd-chelate conjugated 8-arm PEG. (b: 8-arm PEG, c: 8-arm PEG-F, d: 8-arm PEG-FGd$_3$). (e, f) Hydrodynamic radius of 8-arm PEG, 8-arm PEG-F, and 8-arm PEG-FGd$_3$ in R_1 (e) and R_2 (f).

2. Observation on SPM

At the low concentration of 5 mgl/mL, the 8-arm PEG-FGd3 formed particle structure. The fluorescein was located at the core in **core-shell nanoparticles**, which was revealed by spectroscopic analysis.

At the high concentration of 200 mg/mL, the images revealed a **fibrous structure** with a self-assembled continuous lining.

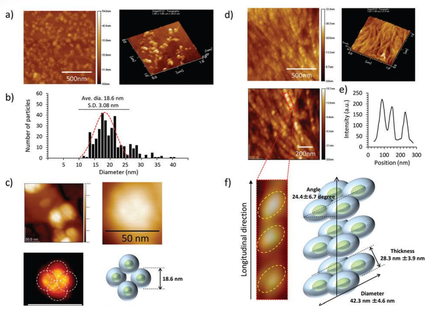


Figure 3. Core–shell nanoparticles, self-assembled tetramers, and supramolecular fibers on SPM images. Polymer concentration is (a-c) 5mg/mL and (d-f) 200 mg/mL.

3. Supramolecular formation process

The 8-arm PEG-FGd3 forms core–shell nanoparticles due to π-stacking interactions of the fluorescein. As the concentration increases, these particles form self-assembled tetramers and fibrous structures. We speculate that the **supramolecular self-assembly of 8-arm PEG-FGd3 occurs in a multi-step process**.

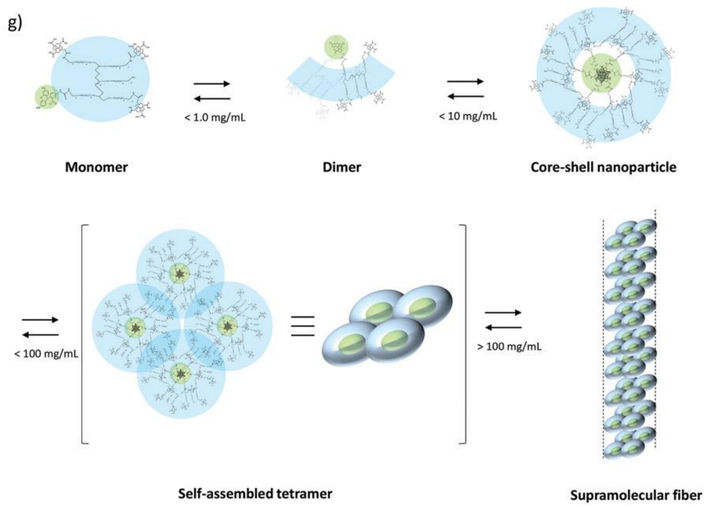


Figure 4. Illustration of the supramolecular fibre formation process.

4. Microvasculature imaging of rat brain on MRI

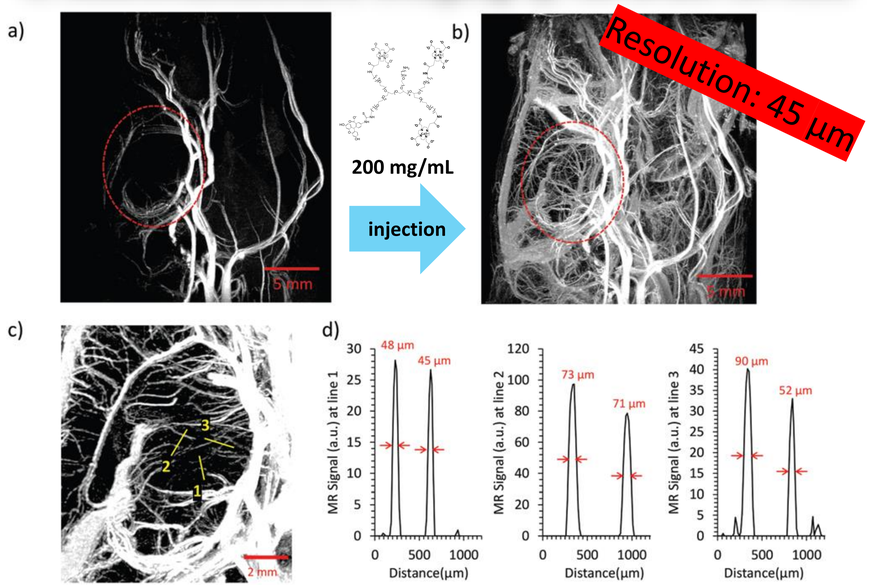


Figure 5. Capillary MR imaging in the rat brain using 8-arm PEG-FGd3. (a and b) Sagittal images of the rat are shown as maximum intensity projections before (a) and after (b) administration of 8-arm PEG-FGd3. The red dotted line indicates the brain region. (c) Volume-rendered MR images of brain regions. Three positions of interest were set as lines 1, 2, and 3. (d) Signal intensity on lines 1, 2, and 3 was plotted against position. The signals were fitted as a Gaussian distribution, and the half-width was used to determine the resolution.

Conclusion

We developed an 8-arm PEG-FGd contrast agent for MR imaging, which enabled us to visualize microvessels and capillaries at a resolution of 45 mm. Our findings also indicated that 8-arm PEG-FGd3 formed self-assembled supramolecular fibres via a multi-step structural transition process. Notably, these agents allow for continuous, in vivo monitoring of capillaries throughout the brain in realtime. As such, they are expected to be highly useful in further studies of brain disease and vascular function.

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