様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 １２ 日現在

機関番号：８４４０４
研究種目：研究活動スタート支援
研究期間：2022～2023
課題番号：２２Ｋ２０５２０

研究課題名（和文）Visualising superfine cerebrovasculature in MRI using supramolecular self-assembly

研究課題名（英文）Visualising superfine cerebrovasculature in MRI using supramolecular self-assembly

研究代表者
Ｓｏｎｉ　Ｒａｇｈａｖ（SONI, RAGHAV）

国立研究開発法人国立循環器病研究センター・研究所・リサーチフェロー

研究者番号：１０９６７８６３

交付決定額（研究期間全体）：（直接経費）　　2,200,000円

研究成果の概要（和文）：本研究では、脳血管系の高解像度イメージングのための自己組織化酸化鉄ナノ粒子ベースの造影剤を作成した。分子量約10kDaの分岐ポリエチレングリコールに酸化鉄ナノ粒子とフルオレセインを結合させた。自己組織化繊維構造は濃度に依存し、静電相互作用によって形成される。長さ120 nm、幅50 nmの自己組織化繊維が25 mg/mlで検出された。さらに、自己組織化造影剤をラットモデルに静脈内投与したところ、従来のMRIイメージングよりも有意な脳微小血管網が観察された。

研究成果の学術的意義や社会的意義
This study demonstrated that self-assemble contrast, when delivered into the circulation, can visualize microvascular networks that conventional MRI imaging cannot, making it an effective new imaging tool for diagnosing cerebral atherosclerosis and stroke.

研究成果の概要（英文）：In this study, we created a self-assembled iron oxide nanoparticle-based contrast agent for high-resolution imaging of the cerebrovasculature. The branched polyethylene glycols having approximately 10 kDa of molecular weight are conjugated with iron oxide nanoparticles and fluorescein. The self-assembled fiber structure is concentration-dependent and formed by electrostatic interaction. Self-assembly fibers measuring 120 nm in length and 50 nm in width are detected at 25 mg/ml. Furthermore, self-assembly contrast administered intravenously in a rat model revealed a more significant cerebral microvascular network than conventional MRI imaging. The research result was presented at several domestic conferences and published in an international journal.

研究分野：Biomaterials and Imaging

キーワード：Nanoparticles　Fluorescein　PEG　Self-assembly　MRI contrast

１．研究開始当初の背景

Magnetic resonance imaging (MRI) has the potential to acquire cerebral vasculature images in high-spatial-resolution. However, MR techniques are limited for visualizing the small cerebral vessels because the signal obtained from a small vessel is weak. In clinical applications, gadolinium (Gd) chelate are used as contrast agents for visualizing cerebral vasculature. However, the Gd chelate diffuses quickly to extravascular tissues while circulating throughout the whole body. As a result, the MR contrast agent concentration in the blood vessel rapidly decreases. Moreover, these small molecule contrast agents exhibit short blood circulation (less than 30 min) and tissue retention time. Therefore, it is difficult to visualize the small vascular network using conventional contrast agents. Researchers have used Gd-chelate conjugated macromolecules such as dendrimers, micelles, polymers, and nanoclusters to enhance circulation time. However, these conjugated contrast agents have difficulty excreting through the urinary tract because of their higher molecular weight.

The iron oxide nanoparticles (IONPs) with size of 10 nm have been clinically approved as reticuloendothelial specific contrast agent. IONPs are promising contrast agents in terms of their high T1/T2 relaxation time and biocompatibility. However, IONPs easily accumulate in the liver and possess shorter circulation time. Therefore, microvessel imaging is not possible with them. Nanoparticles size between ~100 to 200 nm should be optimal to achieve maximum stability and longer circulation time. Our previous study succeeded to show the supramolecular self-assembly of fluorescein/gadolinium-conjugated 8-arm polyethylene glycol (PEG) to prolong blood circulation and enhance the microvessel MR signals. The self-assembled structure is formed by the fluorescein groups stacked with one another via π-electron interaction. Herein, we are trying to synthesis

fluorescein/Fe_3O_4 conjugated PEG that can form supramolecular self-assemble structure for cerebrovascular imaging.

２．研究の目的

The purpose of research for developing a supramolecular self-assemble IONPs/PEG-based contrast agent to visualize microvessels in cerebral vasculature that optimizes the signal in the intravascular space and circulates stably in the blood.

３．研究の方法

The synthesis of 4 arm-PEG-F-IONPs is shown in **Fig. 1A**. Iron oxide nanoparticles (1 mg, 10 nm) were suspended in succinic acid/DMSO solution (4 g in 20 ml) and continuously staring for 3 h to get carboxylic functionalized-IONPs. Fluorescein conjugated PEG (4 arm-PEG, Mw 10k) was added to the Fe_3O_4/DMSO suspension. Finally, the reaction suspension was washed by dialysis and followed by lyophilization to get 4 arm-PEG-F-IONPs.

Hydrodynamic radii were determined by a Malvern Zeta Nanosizer (Malvern Instruments Ltd, Worcester, UK). Briefly, 4 arm-PEG-F-IONPs were dissolved into water at different concentrations from 1.5 mg/ml to 100 mg/ml, to analyze hydrodynamic radii change with concentration.

The self-assembled structures were evaluated using JSPM-5200 apparatus (JEOL Ltd. Japan). An AIO Cantilever (Budget Sensors, Bulgaria) was used in the non-contact mode of atomic force microscopy (AFM). The AFM images were analyzed using ImageJ software.

Brain MRA was performed using a 40-mm inner diameter quadrature volume coil for radiofrequency transmission and signal reception. MRA scans were obtained using a 7 T Horizontal Bore Imaging System (Bruker, Germany).

4．研究成果

4 arm-PEG-F-IONPs were dissolved in water with different concentrations from 1.5 mg/ml to 100 mg/ml and analyzed using dynamic light scattering. Fluorescein conjugation from the aggregation is owing to electrostatic interaction; therefore, the aggregate size continually increases with higher concentration. However, a similar kind of behavior was not observed without fluorescein. The aggregate morphology of 4 arm-PEG-F-IONPs at 25 mg/ml was analyzed using AFM. Aggregate from a fibers structure of length 120 nm and width 50 nm. The proposed formation of fiber structure is shown in **Fig. 1B**.

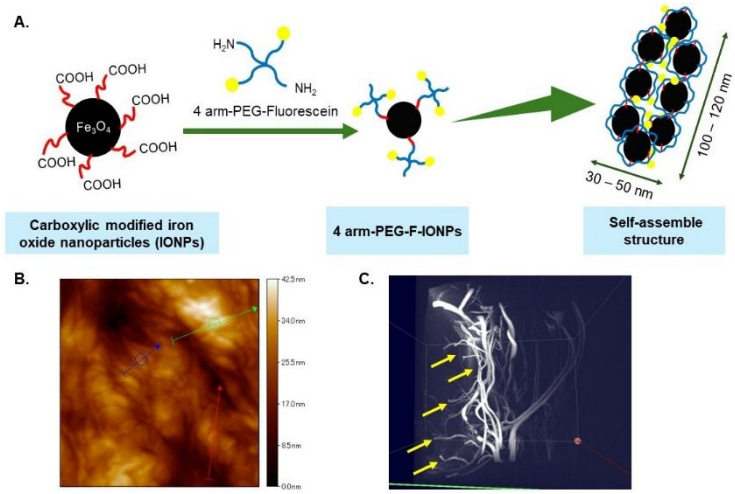

Figure 1. (A) Illustrative representation of synthesis process. (B) Self-assemble fiber structure is confirmed with AFM analysis. (C) Rat brain

Next, we evaluated the resolution of brain microvessels on MRA in **Fig. 1C** include sagittal images of the rat head. Prior to injection of 4 arm-PEG-F-IONPs, we were unable to detect microvessels, although the vessels around the carotid artery and circle of Willis were clearly visible. After injection of 4 arm-PEG-F-IONPs, the signals from microvessels were strongly enhanced. The yellow arrow indicates the contrast enhances microvessels in the cerebral vasculature.

Here, we developed a 4 arm-PEG-F-IONPs contrast agent for MR imaging, which enabled us to visualize microvessels in the cerebral vasculature. Our findings also indicated that 4 arm-PEG-F-IONPs formed self-assembled supramolecular fibers via a multi-step structural transition process. Notably, these agents allow for continuous, in vivo monitoring of microvessels throughout the brain in real-time. As such, they are expected to be highly useful in further brain disease and vascular function studies.

５．主な発表論文等

〔雑誌論文〕　計1件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Mahara Atsushi、Shima Keigo、Soni Raghav、Onishi Ryutaro、Hirano Yoshiaki、Saito Shigeyoshi、Yamaoka Tetsuji	４．巻 230
２．論文標題 In vivo MR imaging for tumor-associated initial neovascularization by supramolecular contrast agents	５．発行年 2023年
３．雑誌名 Colloids and Surfaces B: Biointerfaces	６．最初と最後の頁 113525～113525
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.colsurfb.2023.113525	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計8件（うち招待講演　0件／うち国際学会　7件）

１．発表者名 Raghav Soni, Atsushi Mahara, Tetsuji Yamaoka
２．発表標題 Synthesis of fluorescein/Fe3O4 nanoparticle-conjugated polyethylene glycol for self-assemble MR contrast agent
３．学会等名 44th annual meeting of Japanese Society of Biomaterials (JSB)（国際学会）
４．発表年 2022年～2023年

１．発表者名 Raghav Soni, Tetsuji Yamaoka, Atsushi Mahara
２．発表標題 Synthesis of fluorescein conjugated iron-oxide nanocluster for MR contrast agent
３．学会等名 72nd Society of Polymer Science Japan annual meeting (SPSJ)（国際学会）
４．発表年 2022年～2023年

１．発表者名 Atsushi Mahara, Raghav Soni, Shigeyoshi Saito, Tetsuji Yamaoka
２．発表標題 Self-assembled formation of fluorescein/Gd-chelate-conjugated polyethylene glycol for microvasculature MR imaging
３．学会等名 44th annual meeting of Japanese Society of Biomaterials (JSB)（国際学会）
４．発表年 2022年～2023年

１．発表者名
Atsushi Mahara, Raghav Soni, Shigeyoshi Saito, Tetsuji Yamaoka

２．発表標題
Supramolecular MR contrast agents for visualizing cancer angiogenesis

３．学会等名
72nd Society of Polymer Science Japan annual meeting (SPSJ) (国際学会)

４．発表年
2022年～2023年

１．発表者名
Atsushi Mahara, Raghav Soni, Shigeyoshi Saito, Tetsuji Yamaoka

２．発表標題
Self-assembled formation of fluorescein/Gd-chelate-conjugated polyethylene glycol for microvasculature MR imaging

３．学会等名
71th symposium on macromolecules (国際学会)

４．発表年
2022年～2023年

１．発表者名
Raghav Soni, Tetsuji Yamaoka, Atsushi Mahara

２．発表標題
Self-assemble iron-oxide nanocluster with improved pharmacokinetics

３．学会等名
45th annual meeting of the Japanese society for biomaterials (国際学会)

４．発表年
2023年～2024年

１．発表者名
Raghav Soni

２．発表標題
Self-assemble iron-oxide nanocluster for MR contrast agent

３．学会等名
18th Young Researcher Presentation Meeting JSB Kansai Block (国際学会)

４．発表年
2023年～2024年

１．発表者名
Raghav Soni

２．発表標題
Self-assemble iron-oxide nanocluster for superfine cerebrovasculature MR imaging

３．学会等名
1st annual NCVC symposium

４．発表年
2023年～2024年

〔図書〕　計0件

〔産業財産権〕

〔その他〕
高分子/鉄ナノ微粒子の“弱い”複合体が拓く新たな MRI 診断プローブ～安全性・画像精度の向上
https://main.spsj.or.jp/koho/72n.pdf

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関