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

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研究課題名（和文）Design and application of short chain fatty acid nanoparticles for cancer and obesity therapeutics

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研究代表者

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交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：本研究では、短鎖脂肪酸（プロピオン酸、酪酸）を基盤の高分子自己組織化薬（それぞれPNP,BNP）を新たに設計し、放射線治療の保護剤、及び肥満、非アルコール性脂肪肝炎、糖尿病の治療薬として応用展開した。B16F10の担がんマウスモデルに本高分子薬を投与してから放射線を照射すると、放射線保護剤として機能することを明らかにした。また、短鎖脂肪酸の薬物動態を本高分子薬により改善することで、肥満、非アルコール性脂肪肝炎、糖尿病の治療効果も得ることができた。

研究成果の学術的意義や社会的意義
Clinical application of short-chain fatty acids (SCFA) is limited due to their poor pharmacokinetics. Here, we succeeded in improving their pharmacokinetic properties by delivering them as a prodrug nanoparticle, resulting in a higher therapeutic effect in the several disease.

研究成果の概要（英文）：We successfully synthesized base polymer PEG-b-PVRs (R=propionic, butyric acid) and prepared stable nano-sized short-chain fatty acid nanoparticles (SCFA) (PNP and BNP). We confirmed anti-cancer effect of PNP and BNP in a B16F10 metastasis melanoma model. We also confirmed that by single administration of BNP 24 h prior to irradiation, sensitizes B16F10 subcutaneous tumors in mouse model for the radiation therapy, whereas low molecular weight (LMW) butyric acid had no effect at lower dose. We also confirmed improvement in disease symptoms in in high fat diet-induced obesity and non-alcoholic hepatitis model. Similarly, in diabetic db/db mice, we confirmed that BNP (free drinking) and the conventional exenatide (intraperitoneal) improved the diabetic symptoms such as glucose utilization as compared to LMW SCFA groups. We also observed that by intraperitoneal route, butyric acid released by BNP has higher bioavailability than LMW butyric acid, confirmed by in vivo imaging and LCMS/MS.

研究分野：Biomaterial Sciences

キーワード：Short-chain fatty acid　Polymeric micelle　Self assembling　Controlled release　Cancer　Obesity　Non-alcoholic hepatitis　Diabetes

１．研究開始当初の背景

Short chain fatty acids (SCFAs), namely, acetic acid, butyric acid, and propionic acid, are metabolites produced by gut bacteria by the fermentation of indigestible polysaccharides. SCFAs have been widely reported to exert health benefiting effects and have shown improvement in diseases such as cancer, colitis, diabetes, non-alcoholic hepatitis, and obesity in small animals. However, it should be noted that because SCFAs are low molecular weight (LMW) compounds their therapeutic effects are often accompanied with adverse effects attributed to their poor pharmacokinetic properties such as fast renal clearance and non-specific distribution. It was reported that clinically a small number of patients suffering from Crohn's disease were daily administered with a high dose of butyrate (4g/d), of which 69 % responded to treatment (*Aliment Pharmacol Ther. 2005 Nov 1;22(9):789-94*). Administration of such high dose of SCFAs daily maybe required due to their poor pharmacokinetic property. In this line, we need a suitable drug delivery system which can control the availability of LMW SCFAs for longer time and at the same time do not exert adverse effects. In this work, we aimed to improve the pharmacokinetic property of SCFAs by delivering them using our delivery system: PEG-b-poly(vinyl ester), which self-assembles into nano-sized micelle ($Nano^{SCFA}$) in physiological condition. SCFAs are chemically conjugated to polymer backbone by ester linkage so their release is highly controlled by presence of hydrolyzing enzymes in the intestine, liver, and tumor. With this design, we succeeded in controlling the pharmacokinetic property of SCFA which resulted in higher therapeutic effect as compared to LMW SCFA-treated group in several disease mouse models. We observed improved therapeutic effect in cancer (B16F10), obesity, diabetes, non-alcoholic hepatitis and liver fibrosis, and as a radio-sensitizer for radiation-based cancer therapy. LMW SCFAs on the other hand exerted adverse effects due to rapid spike in systemic concentration which masked its therapeutic effect. Our delivery system will be very useful in future to deliver different types of SCFAs for their clinical use to cure several diseases as compared to LMW SCFAs.

２．研究の目的

Objective of our study is to prepare a delivery system for short chain fatty acids, particularly propionic and butyric acid to improved their pharmacokinetic properties and confirm their improved efficacy in cancer, non-alcoholic hepatitis, diabetes, and obesity as compared to low molecular weight SCFAs.

３．　研究の方法

3.1 Synthesis and characterization of PEG-b-PVRs: PEG-b-PVRs (R=Propionic, Butyric acid) were prepared via RAFT polymerization of vinyl ester monomers using CH_3O-PEG-$SC(=S)OCH_2CH_3$ as a polymer chain transfer agent. Successful synthesis of intermediate and final polymers was confirmed by proton nuclear magnetic resonance and gel permeation chromatography. Obtained polymers were dissolved in DMF and dialyzed against water to form suitable size SCFA nanoparticles due its amphiphilic characters (Fig.1). The nanoparticle diameter and polydispersity index were determined by dynamic light scattering.

3.2 Evaluation of therapeutic efficacy and adverse effects of $Nano^{SCFA}$ in a mouse cancer model

Experimental metastasis model: C57BL/6J (male, 7-8 weeks) were randomly divided into following six groups: Healthy (water), Tumor control (water), BNP, PNP (30 mM), butyric acid, and propionic acid (30 μM). The samples were available as *ad libitum* and started one day prior to inoculation of melanoma cells B16F10 to the termination day. B16 F10 cells (2.5×10^5/200 μL) were injected via tail vein to prepare a metastasis model. On 11^{th} day, post cell inoculation, mice were sacrificed and superficial lung tumors were counted.

Radiation therapy: A subcutaneous tumor model was prepared in a C57BL/6J strain mice (male, 7-8 weeks) by injecting B16 F10 cells (0.76 x 10^6/100 µL) into right flank. 9 days post cell inoculation, mice were randomly divided into following groups: healthy, tumor, tumor + irradiation (IR), BNP + IR (200 mg/kg), butyric acid + IR (200 mg/kg). When the tumor reached ca. 146 ± 18 mm^3 in size, samples were injected intraperitoneally. 24 h later, tumor area was irradiated (5 Gy, Al + Cu filter). On day 6 after irradiation, mice were sacrificed and major organs and blood were collected for further analysis.
Diabetes: Samples were available as free drinking in C57BL/6J, db/db mice (male, 7-8 weeks): control (water), BNP, PNP, butyric acid, propionic acid (60 mM), and exenatide (1-2 µg, i.p.). At 37th day, a glucose tolerance test was conducted. Difference between glucose concentration at 0 h and 1 h was used to plot the graph (*Shashni et al., Biomaterials 275 (2021) 120877*).
Obesity: C57BL / 6J mice (male, 4 week) were fed with high fat diet (60% ultra-high fat feed) and BNP and PNP (5 mg/ml) were available as free drinking.
Non-alcoholic hepatis (NASH) and liver fibrosis: C57BL/6J (6 week) mice were fed on choline-deficient, L-amino acid-defined, high-fat diet for 2 months and nanoparticles and LMW SCFA (100 mM) were available as free drinking.

3.3 Pharmacokinetic study of Nano^{SCFA}: Biodistribution of SCFA release from nanoparticles was determined by ELISA and LC-MS/MS and polymer itself (conjugated with a fluorescent TAMRA dye) was evaluated by in vivo imaging system.

4. 研究成果

4.1 Synthesis and characterization of PEG-b-PVRs and Nano^{SCFA} preparation

We prepared a metabolizable amphiphilic block copolymer which contains hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(vinyl SCFA ester) (propionic (PR) and butyric (Bu) acids); PEG-b-PV(Pr) and PEG-b-PV(Bu), respectively by RAFT polymerization (Fig. 1A), which were confirmed by ^{1}H NMR and GPC. The base polymer had 64.1 and 55.6 units of vinyl propionate and vinyl butyric acid, respectively. We obtained 86.6% and 87.3% yield for PEG-b-PV(Pr) and PEG-b-PV(Bu), respectively. Due to amphiphilic character of the polymers, they formed uniform sized nanoparticle 37.0 nm (PNP) and 40.3 nm (BNP), respectively with polydispersity index < 0.09, indicating uniform size (*Shashni et al., Biomaterials 275 (2021) 120877*) (Fig. 1B and 1C). This was suitable for us to proceed for *in vitro* and *in vivo* application.

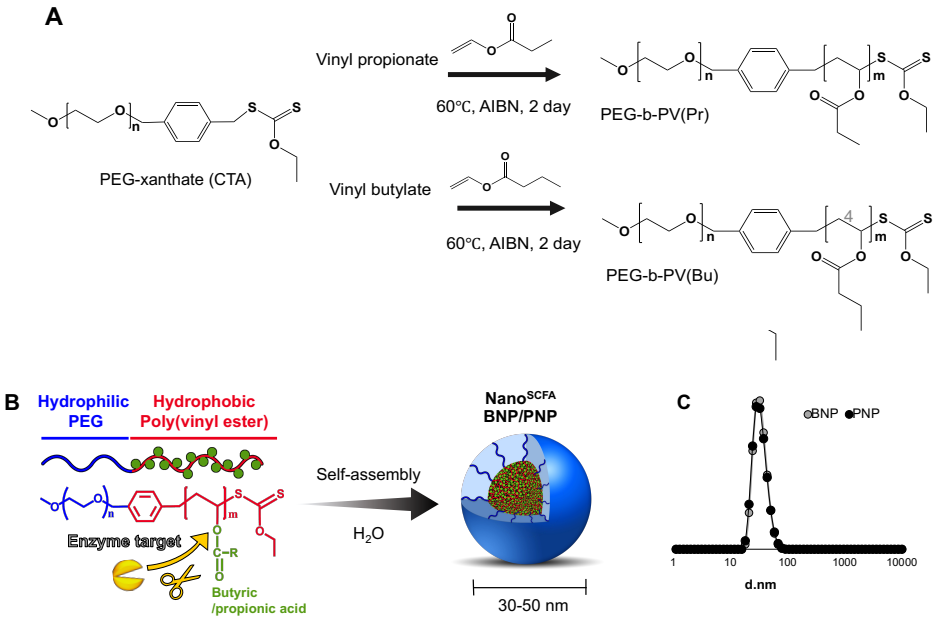

Fig 1. (A) Synthesis scheme of base polymer PEG-b-PV(SCFA). (B) Illustration showing the formation of Nano^{SCFA} based on amphiphilic character of polymers. (C) Size distribution of BNP and PNP measured by DLS (*Shashni et al., Biomaterials 275 (2021) 120877*).

4.2 Experimental metastasis model: Treatment of BNP and PNP decreased the metastatic nodules of B16 F10 in the lungs as compared to tumor control and LMW SCFA, which corroborated with tumor area in lung section stained with H and E stain. On the other hand, LMW SCFA did not exert any anti-tumor effects, possibly due to low concentration. However, villi of small intestine were observed to be shorter in only propionic acid treated group, whereas BNP and PNP did not exert any damage to intestine.

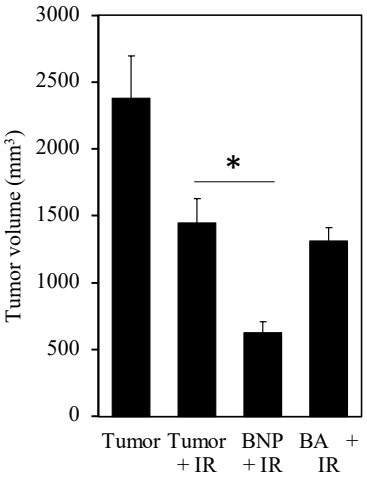

Fig 2. Tumor volume at the experimental end day. P<0.05 "*".

4.3 Radiation therapy: We confirmed that single administration of BNP resulted in sensitizing of B16F10 tumors for the radiation therapy. Average tumor volume in BNP-treated group (625 ± 82.8 mm^3) was significantly lower than butyric acid treated group (1313.5 ± 94.8 mm^3), irradiated group (1444.0 ± 183.2 mm^3) and tumor control itself (2377.0 ± 319.8 mm^3) (Fig. 2). Down-regulation of several proliferation proteins were observed in BNP treated tumors such as Cox-2.

4.4 Diabetes: Anti-diabetic effect of SCFA nanoparticle was conducted in db/db mice. The glucose tolerance test conducted on 37th day of treatment confirmed that BNP and exenatide had the highest effect for glucose utilization, whereas LMW SCFA group showed negligible effect (Fig. 3A). PNP showed similar tendency as that of BNP, however no significant difference was observed (*Shashni et al., Biomaterials 275 (2021) 120877*). Nano^{SCFA} did not exert any adverse effects. Negligible change was observed in the body weight, organ weight, total blood count (platelets, RBC, hemoglobin, and WBC), and organ function markers (ALT, AST, BUN, and CRE) (Fig. 3B). Whereas, LMW propionic acid and exenatide-treated mice showed increased platelets and liver and kidney function markers. In addition, villi of small intestine and colon were also affected by LMW group SCFA and exenatide (Fig. 3C).

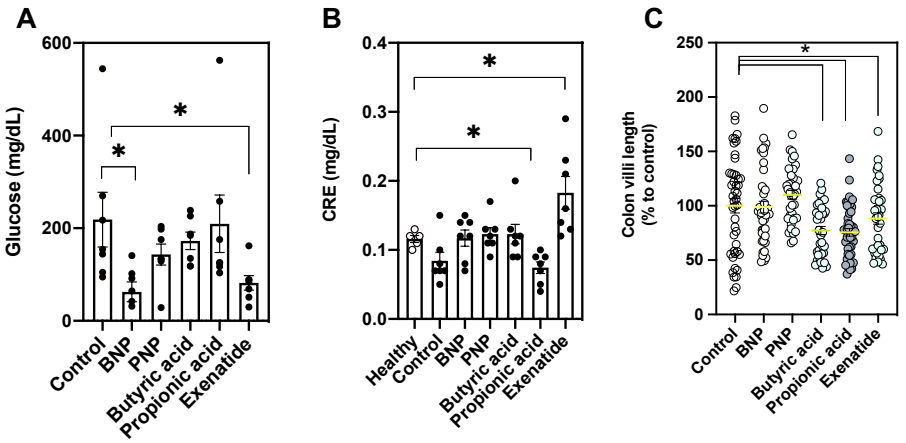

Fig 3. (A) BNP and exenatide treatment improved glucose utilization as confirmed by glucose tolerance test. (B) Kidney damage marker: creatinine level in plasma. (C) Villi

length in colon. P<0.05 "*" was considered significant (*Shashni et al., Biomaterials 275 (2021) 120877*).

4.5 Non-alcoholic hepatis (NASH) and liver fibrosis: PNP and BNP treatment significantly decreased accumulation of lipid and formation fibrotic collagen in the livers which corroborated with decreased hepatic damage maker (ALT and AST), and white blood cells in blood. Whereas LMW SCFAs had negligible effect on the improvement of fatty liver disease.

4.6 Obesity: Free administration of BNP and PNP while mice are fed with high fat diet (60% ultra-high fat feed), prevented weight gain significantly as compared to control high fat diet fed mice (Fig. 4).

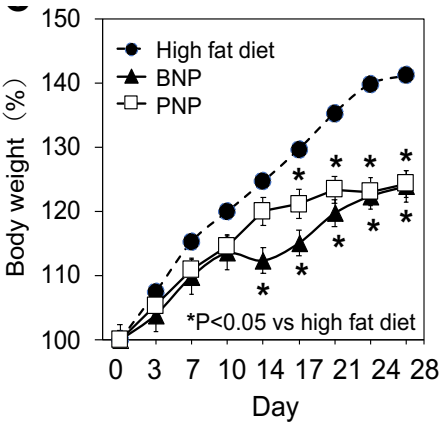

Fig 4. Nano^{SCFA} prevents weight gain in the obese mice. P<0.05 "*"

4.7 Pharmacokinetic study of Nano$^{SCFA:}$ Intraperitoneal administration of fluorescently (TAMRA) labeled BNP in tumor bearing mice confirmed that nanoparticles accumulate in tumors by enhanced retention and permeation effect evaluated by in vivo imaging. Fluorescent signals of BNP were observed in the extracted tumors and plasma until 3 days indicating high bioavailability. We also observed released butyric acid from BNP until 40 h post administration, whereas LMW butyric acid was cleared from the blood within 0.5 h as measured by LC-MS/MS. Similar tendency of biodistribution was observed in radio-labelled I^{125} phenyl butyrate nanoparticle in the tumor-bearing mice. When administered orally, BNP resided in GI tract for 2 days and negligible presence was observed in blood (Fig. 5). However, butyric acid released by BNP was observed at a higher level in plasma as compared to LMW butyric acid (*Shashni et al., Biomaterials 275 (2021) 120877*).

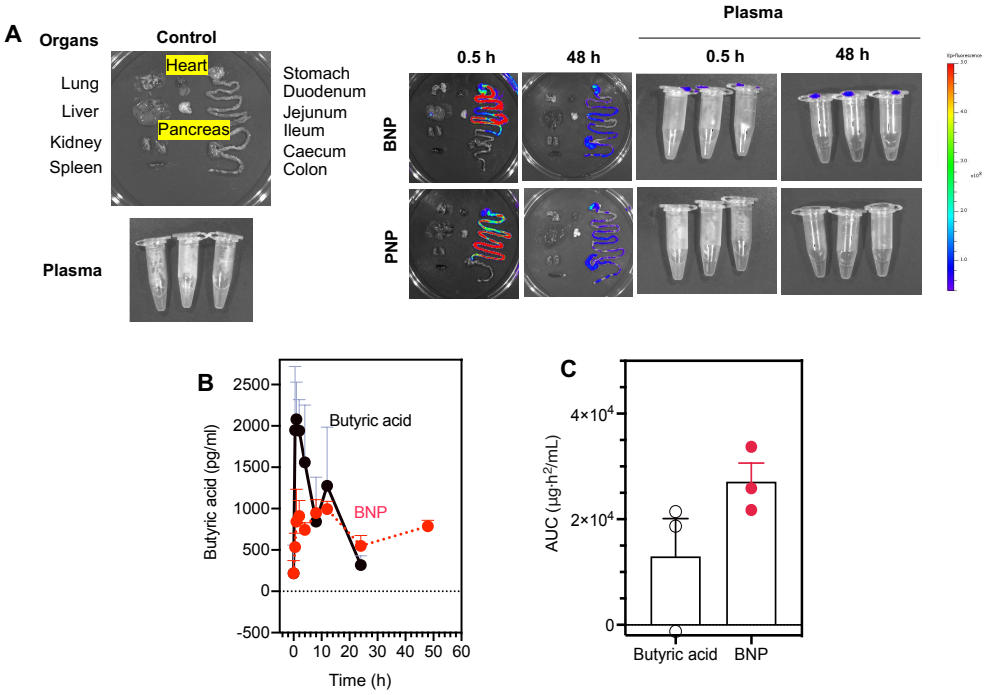

Fig 5. (A) TAMRA-labelled BNP and PNP were administered orally and their fluorescent signals were observed by in vivo imaging system in the organs and plasma. (B) Butyric acid in plasma administered with BNP as assessed by ELISA. (C) Area under curve for the bioavailability graph (B) (*Shashni et al., Biomaterials 275 (2021) 120877*).

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　5件／うちオープンアクセス　1件）

１．著者名 Mei Ting、Shashni Babita、Maeda Hiroshi、Nagasaki Yukio	４．巻 259
２．論文標題 Fibrinolytic tissue plasminogen activator installed redox-active nanoparticles (t-PA@iRNP) for cancer therapy	５．発行年 2020年
３．雑誌名 Biomaterials	６．最初と最後の頁 120290～120290
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.biomaterials.2020.120290	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

１．著者名 Shashni Babita、Nishikawa Yuji、Nagasaki Yukio	４．巻 269
２．論文標題 Management of tumor growth and angiogenesis in triple-negative breast cancer by using redox nanoparticles	５．発行年 2021年
３．雑誌名 Biomaterials	６．最初と最後の頁 120645～120645
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.biomaterials.2020.120645	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

１．著者名 Shashni Babita、Nagasaki Yukio	４．巻 11
２．論文標題 Newly Developed Self-Assembling Antioxidants as Potential Therapeutics for the Cancers	５．発行年 2021年
３．雑誌名 Journal of Personalized Medicine	６．最初と最後の頁 92～92
掲載論文のDOI（デジタルオブジェクト識別子） 10.3390/jpm11020092	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Shashni Babita、Tajika Yuya、Nagasaki Yukio	４．巻 275
２．論文標題 Design of enzyme-responsive short-chain fatty acid-based self-assembling drug for alleviation of type 2 diabetes mellitus	５．発行年 2021年
３．雑誌名 Biomaterials	６．最初と最後の頁 120877～120877
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.biomaterials.2021.120877	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

１．著者名 Kim Ahram、Yonemoto Chiaki、Feliciano Chitho P.、Shashni Babita、Nagasaki Yukio	４．巻 17
２．論文標題 Antioxidant Nanomedicine Significantly Enhances the Survival Benefit of Radiation Cancer Therapy by Mitigating Oxidative Stress‐Induced Side Effects	５．発行年 2021年
３．雑誌名 Small	６．最初と最後の頁 2008210～2008210
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/smll.202008210	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17件（うち招待講演　3件／うち国際学会　2件）

１．発表者名 Babita Shashni and Yukio Nagasaki
２．発表標題 Self-assembling short-chain fatty acid nanomedicine for the treatment of diabetes
３．学会等名 The 30th Annual meeting of MRS-J
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１．発表者名 Babita Shashni, Yuya Tajika, Yukio Nagasaki
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３．学会等名 3rd GLowing Polymer Symposium in KANTO (GPS-K 2020)
４．発表年 2020年

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２．発表標題 短鎖脂肪酸分子組織化薬の設計
３．学会等名 第 49 回医用高分子シンポジウム (Cancelled due to COVID-19)
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２．発表標題
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３．学会等名
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４．発表年
2020年

１．発表者名
Yuya Tajika, Babita Shashni, Yuji Nishikawa, Yukio Nagasaki

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２．発表標題
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４．発表年
2021年

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Metabolizable PEG-b-poly(vinyl ester) based self-assembling nanoparticle for the therapeutics of various diseases

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２．発表標題
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2021年

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２．発表標題
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４．発表年
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１．発表者名
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２．発表標題
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４．発表年
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２．発表標題
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３．学会等名
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４．発表年
2021年

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An enzyme-metabolizable short-chain fatty acid polymer for therapeutics of various disease

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Materials Research Meeting 2021 (MRM2021)

４．発表年
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2021年

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２．発表標題
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２．発表標題
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３．学会等名
The 69th Symposium on Macromolecules, SPSJ

４．発表年
2020年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

The 3rd GLowing Polymer Symposium in KANTO (GPS-K 2020), November 28th, 2020 (On-line symposium). Best presentation award.

The 69th Symposium on Macromolecules SPSJ, September 16th - 18th 2020 (Online). Publicity award.

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