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研究課題名（和文）Establishing design principles for spray dried nanofibrillar supraparticles towards modular pulmonary drug delivery systems

研究課題名（英文）Establishing design principles for spray dried nanofibrillar supraparticles towards modular pulmonary drug delivery systems

研究代表者

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研究成果の概要（和文）：超微粒子は、より小さな粒子の構成要素から構成される粒子構造です。我々の研究結果では、タンパク質ナノフィブリルをナノ粒子（直径 1/10000 ミリメートル未満）と混合し、スプレー乾燥技術を使用して構造化させると、しわ状の超微粒子を作製できることが示されています。より大きなナノ粒子では、しわ状の超微粒子を作製するためにより多くのナノフィブリルが必要でしたが、構成要素の柔らかさとアスペクト比により、ナノフィブリルが超微粒子の形状を変更する傾向が低下しました。ナノフィブリルは、薬物が超微粒子構造に組み込まれた場合、しわのある形状を作製するのに効果的でした。

研究成果の学術的意義や社会的意義
超微粒子は、製薬業界や化学業界など、多くの有望な用途があります。我々の研究結果は、産業関連の粒子形成技術（スプレー乾燥）を使用してタンパク質ナノフィブリルを超微粒子に組み込むことで、超微粒子の構造を変更する方法に関する知識を広げる。材料の性能は一般にその構造（構造と特性の関係）と関連しているため、我々の研究結果は、様々な用途で超微粒子の性能を変更するためにすぐに採用できる可能性があります。我々の研究結果は、同様の有用性を持つ可能性のある他のナノフィブリル構成要素に関するさらなる研究の動機にもなりうる。

研究成果の概要（英文）：Supraparticles are particle constructs composed of smaller particle building blocks. Our results show that protein nanofibrils can be used to create supraparticles with crumpled shapes when mixed with nanoparticles (diameter below 1/10000 of a millimeter) and assembled using the spray drying technique. Larger nanoparticles required more nanofibrils for the creation of crumpled supraparticles, whereas building block softness and aspect ratio decreased the propensity of the nanofibrils to modify the supraparticle shape. The nanofibrils remained effective in creating crumpled shapes when drugs were incorporated within the supraparticle structure.

研究分野：Materials science

キーワード：Supraparticles　Spray drying　Amyloid nanofibrils　Silver nanowires　Silica nanoparticles　Phytoglycogen　Crumpling

１．研究開始当初の背景

Millimetric and micron-sized particle constructs called supraparticles (SPs) composed of nanoparticles have found many promising applications in diverse fields such as drug delivery, catalysis, and sensing. Generally, particle morphology has a large influence on their material properties and performance in the target application. Therefore, methods influencing the nanoparticle organization and the shape of the SPs are broadly applicable and possess a considerable general interest.

In recent years, high aspect ratio cellulose nanofibrils have been used to improve the mechanical properties of SPs owing to their ability to form highly entangled fibril networks. However, their potential to influence the SP morphology, such as creating crumpled SP shapes, has not been investigated. This study was initiated to explore this overlooked aspect of the SP assembly of nanoparticles and nanofibrils using an industrially-relevant spray drying method by employing protein-based nanofibrils that may have potential advantages in drug delivery applications over polysaccharide-based nanofibrils, such as better bioclearance, owing to their supramolecular organization.

２．研究の目的

The objectives of this study were twofold. Firstly, we set out to understand the interplay between different SP building blocks, namely nanoparticles and nanofibrils, in the SP assembly process occurring during spray drying. Specifically, our objective was to elucidate how nanoparticle size, shape, and surface chemistry influence the SP morphology (e.g. crumpling) with proteinaceous amyloid nanofibrils (ANFs) by varying the precursor suspension composition. Secondly, we set out to understand whether ANFs influence the disassembly, drug release, and aerodynamic properties of the SPs in order to yield insights into their potential use in pulmonary drug delivery applications.

３．研究の方法

ANFs were synthesized from beta-lactoglobulin (βLG) using an established literature protocol. Commercial silica nanoparticles (SiNPs) with diameters of 10, 50, and 100 nm were selected as the main model nanoparticle building blocks. Commercial sweet-corn phytoglycogen (PG) was chosen as the model soft polysaccharide nanoparticle due to the following reasons: (i) its approximately 50 nm mean diameter, which facilitated comparisons with hard SiNPs, (ii) the availability of the host research group's past characterization results and experiences employing PG in spray drying applications, and (iii) the growing importance of glycogen in nanosciences. Silver nanowires (AgNWs) were synthesized using an established polyol method and used as the rod-like hard model building block. Carvedilol was used as the model drug in drug encapsulation and release studies.

Building blocks were characterized using hydrodynamic size, zeta potential and/or by their physical dimensions. ANF length and thickness were measured using transmission electron microscopy (TEM). The spray drying parameters were kept constant for all formulations. The total volume fraction of solids was kept constant at 0.0005 to facilitate comparisons between different morphologies. This value was chosen based on the size of spray-dried SiNP SPs that yielded SPs with diameters ranging around 1 to 5 μm, which is attractive for pulmonary drug delivery applications intended to study in the second part of the project. The efficacy of ANFs to instigate particle crumpling was assessed using scanning electron microscopy (SEM) by measuring the thickness of the final crumpled surface undulations (H) and the overall particle size based on two estimated principal directions (a and b) of the particles on the SEM sample holder (Fig. 1). Protein secondary structure of the ANFs before and after spray drying was analyzed with Fourier-transform infrared spectroscopy (FTIR). Drug-loaded SPs were characterized with SEM, FTIR, and powder X-ray diffraction. Drug release kinetics were analyzed with high-performance liquid chromatography.

4．研究成果

The results of the first part, namely the morphological modification of spray-dried SPs with ANFs, have been published in a peer-reviewed journal (Ref. ①), whereas another publication is under preparation for the second part of the grant proposal concerning the results obtained by comparing the drug encapsulation and drug release properties of the ANF-infused SPs.

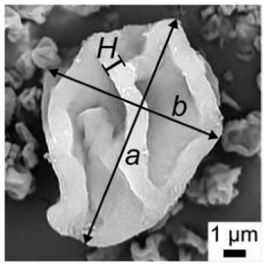

Fig. 1. SEM image of a crumpled SP and the definitions of measured parameters used to calculate the shell thickness ($h = H/2$) and estimated particle size using a and b. Reused from Ref. ① with permission. © 2024 Wiley‐VCH GmbH.

The influence of ANFs on the morphology of spray-dried SiNP SPs was clearly evidenced by SEM (Fig. 2). For each SiNP size, the incorporation of ANFs transformed the SPs into highly crumpled shapes as long as sufficient ANF loading was used. This critical ANF volume fraction followed linearly with the SiNP diameter, whereby roughly 1 vol% more ANFs was required to instigate crumpling for every 10 nm increase in the SiNP diameter. Therefore, more ANFs were needed to crumple SPs containing larger nanoparticles. Based on SEM measurements, the density of ANF networks in the SP increased with SiNP size and decreased with SiNP volume fraction. The results further showed that an increase in the ANF volume fraction led to a decrease in the shell thickness of the SP undulations as shown for 10 nm SiNP SPs in Fig. 3. These results suggest that ANFs promote the structural arrest of the suspension during spray drying and thereby cause a buckling transition that leaves the surfaces decorated with undulating topographical features.

TEM imaging of SiNP-ANF SPs did not show presence of large building block concentration heterogeneities, which indicates that ANFs and SiNPs are mixed well in the final SP structure. FTIR of the SPs showed red and blue-shifted features in the amide I bands typically used to discern protein secondary structures corresponding to β-sheets, turns, α-sheets, and random coils, but the exact quantitative nature of these changes remained inconclusive. TEM imaging of reconstituted ANF SPs showed short fibrillar fragments suggesting that the fibrillar organization is preserved to some extent after spray drying.

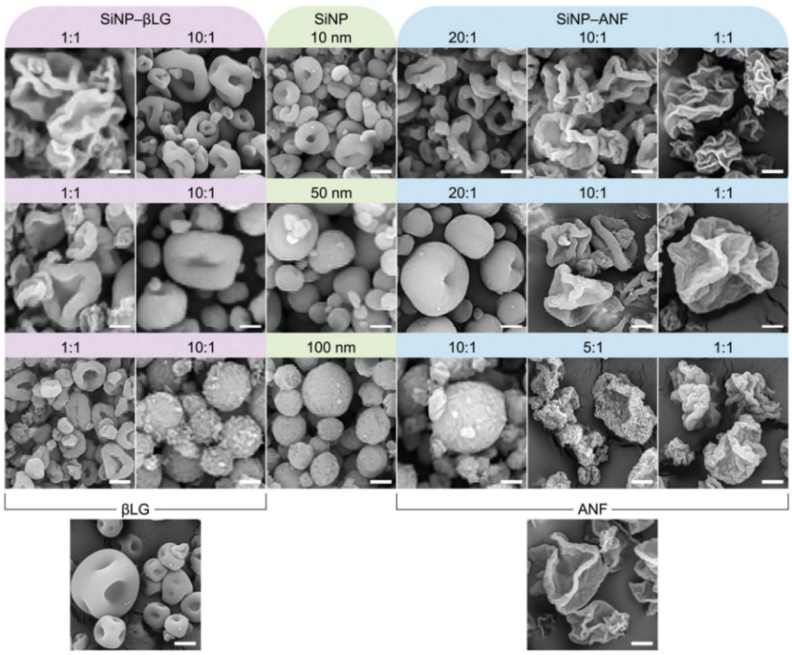

Fig. 2. SEM images of spray-dried SPs with varying volume ratios of SiNPs, βLG, and ANFs. The bottom images show SPs prepared purely from βLG or ANFs. Reused from Ref. ① with permission. © 2024 Wiley‐VCH GmbH.

Influence of ANF length was investigated by subjecting the ANF suspensions to bath ultrasonication. Hydrodynamic shear forces generated during ultrasonication have been previously shown to cause ANF breakup. TEM imaging showed clear fragmentation of the ANFs with ultrasonication treatment time leading to decrease in the ANF length, which was supported by decreased shear viscosity of the suspensions. However, the fragmentation of ANFs, i.e., the decrease in ANF length, did not

influence the SP morphology greatly. Only with 100 nm SiNPs, the shell thickness-to-SP size ratio increased somewhat (Fig. 4), which was likely related to a diminished propensity of the ANFs to form entangled networks as they become sufficiently short relative to the nanoparticle size to link neighboring nanoparticles into a cohesive arrested structure during spray drying.

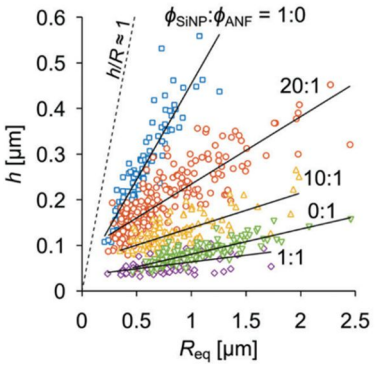

Fig. 3. Measured shell thickness (h) of 10 nm SiNP-ANF SPs for different SiNP:ANF volume ratios as a function of estimated particle size (R_{eq}) calculated from a and b shown in Fig. 1. Reused from Ref. ① with permission. © 2024 Wiley - VCH GmbH.

ANFs retained their ability to instigate crumpled SP morphologies when incorporated with soft nanoparticles, namely PG. When compared to roughly similar-sized 50 nm SiNPs, the crumpling transition was shifted to higher ANF volume fractions. This suggests that soft nanoparticles might have different assembly dynamics during the consolidation. Also, notable differences were found between the monomer form (βLG) and ANFs, which points to the importance of the nanofibrillar organization of ANFs in creating crumpled SP morphologies, and therefore, to the processes involved in forming structurally arrested nanoparticle assemblies during spray drying.

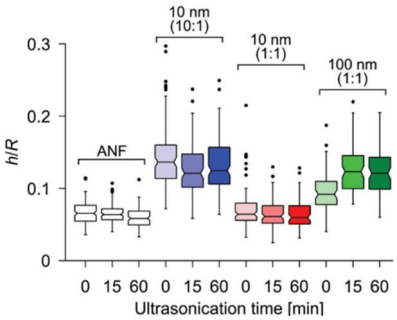

Fig. 4. Notched box plots of the normalized shell thickness (h/R) distribution as a function of ultrasonication time as SiNP diameter and volume ratio SiNP:ANF are varied. Reused from Ref. ① with permission. © 2024 Wiley - VCH GmbH.

The rod-like AgNWs were subjected to tip sonication to decrease their length to a more suitable range for spray drying. The AgNW content in the precursor was selected such that on average roughly few AgNWs per contained within each SP in order to slightly perturb the SP structure formation. When AgNWs were used in conjunction with SiNPs, the efficacy of ANFs to promote crumpled SP morphologies was decreased (Fig. 5). SEM imaging showed that AgNWs were aligned within the smaller SP fraction, which was not greatly affected by the presence of ANFs compared with βLG. For larger SP fraction, crumpled and contorted morphologies were observed instead of toroidal morphologies. However, the difference between ANFs and βLG was not discernible in SiNP-AgNW SPs, which indicates that AgNWs had a greater influence on the final SP morphology.

The second part of project was completed within the final fiscal year and part of the data analysis is still ongoing. Crumpled SP morphologies were also observed after incorporating a model drug carvedilol into the spray drying formulation with 10 or 100 nm SiNPs with a loading chosen below the theoretical inter-nanoparticle pore volume remaining after accounting for a random packing of SiNPs in the SP (Fig. 6). Preliminary data analysis results from drug release studies suggest that ANFs do not greatly influence the release kinetics when drugs were

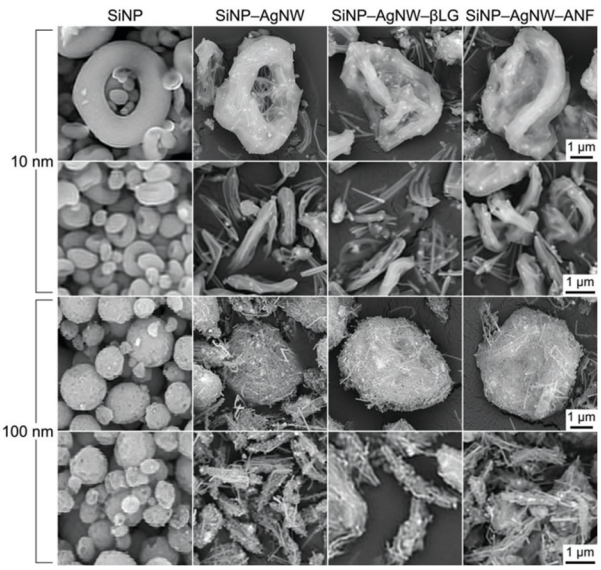

Fig. 5. SEM images of SPs containing AgNWs. Large (small) particle fraction is shown on the top (bottom) row for both SiNP diameters indicated on the left. Reused from Ref. ① with permission. © 2024 Wiley - VCH GmbH.

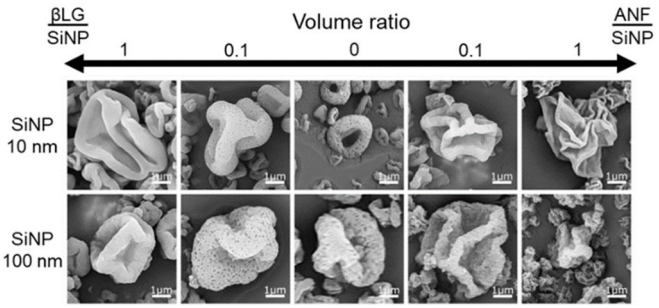

Fig. 6. SEM images of drug-loaded SPs with varying compositions as indicated.

encapsulated in the interstitial pore space of the nanoparticles. Detailed data analysis results of powder X-ray diffraction, FTIR, SEM, and drug release kinetics will be reported later.

Initially, polysaccharide nanoparticles were proposed as the main model building blocks for this study. However, they were replaced with SiNPs due to the commercial availability of SiNPs in many particle sizes with low polydispersity, while possessing a similar hydroxyl-rich surface chemistry, in order to improve the scientific quality of the results as the assembly process was expected to have a considerable size-dependent character, therefore maximizing building block uniformity was of considerable importance for the main model building block. Furthermore, some deviations were taken from the proposal due to time and financial constraints. Owing to the global supply chain disruptions cause by COVID-19 in 2021 and 2022, material procurement was delayed, which postponed the start of experiments requiring ANFs. The planned Andersen cascade impactor experiments used to study the aerodynamic properties of the SPs were omitted as obtaining ANF-infused SPs in sufficient quantities for this study proved to be prohibitively costly and time-consuming. In future studies, this financial cost could be partly alleviated by using more affordable proteins for the ANF synthesis such as bovine serum albumin or lysozyme. Also, different surface modifications the nanoparticles were not pursued due to time constraints of the project. Similarly, employing the polyphenolic particles, used by the principal investigator in his earlier research work cited in the grant proposal, turned out to be too resilient to further disintegration to obtain the required nanosized dimensions, while controlling the particle size synthetically posed purity concerns. To circumvent these issues in a timely manner, AgNWs were selected as the rod-like model nanoparticle.

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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　0件／うちオープンアクセス　0件）

１．著者名 Kamarainen Tero、Nakayama Yuzuki、Uchiyama Hiromasa、Tozuka Yuichi、Kadota Kazunori	４．巻 -
２．論文標題 Amyloid Nanofibril‐Assisted Spray Drying of Crumpled Supraparticles	５．発行年 2024年
３．雑誌名 Small	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/smll.202309645	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 －

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１．発表者名 Tero Kamarainen
２．発表標題 Spray-drying flexible proteinaceous nanofibrils with hard and soft nanoparticles towards the morphological engineering of pulmonary drug delivery systems
３．学会等名 The 3rd International Conference of Lignocellulose (ICONLIG) in Conjunction with The 15th International Symposium of IWoRS（国際学会）
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２．発表標題 Morphological Modification of Spray-Dried Supraparticles with Amyloid Nanofibrils
３．学会等名 Spray-Drying Symposium（国際学会）
４．発表年 2024年

１．発表者名 Tero Kamarainen
２．発表標題 Increasing heterogeneity of supraparticles via spray drying
３．学会等名 粉体工学会2022年度秋期研究発表会
４．発表年 2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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〔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

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