Annual Report for FY2023-2024

Laboratory of nanofiber technology

Gifu Pharmaceutical University

岐阜薬科大学　ナノファイバー創剤学寄附講座

年次報告書 2023年–2024年

岐阜薬科大学 × MITSUBISHI CHEMICAL GROUP

三菱ケミカル株式会社

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愛知医科大学
解剖学講座
助教・呼吸器外科医

大久保　友人
Okubo Tomohito

私は呼吸器外科医として、主に肺癌や胸腔内感染症、嚢胞性肺疾患を対象に治療を行っております。私の診療分野では、喫煙などの影響により肺組織が脆弱化している患者さんが多く、肺や気管支に生じた瘻孔が治癒せず、治療に難渋する症例にしばしば直面します。

肺や気管支といった空気を含む臓器に瘻孔が形成されると、呼吸に伴って瘻孔を経由する気流が創傷治癒を妨げるため、ごく小さなピンホール状の穴であっても閉鎖には時間を要します。その結果、感染症などの合併症が生じ、入院が長期化することで、患者さんと医療従事者の双方が大きな負担を抱える状況となっています。

現在、肺や気管支に生じた難治性瘻孔に対しては、瘻孔部位を含めた臓器の切除が不可能な場合、患者さんご自身の血流が豊富な脂肪や筋肉組織を瘻孔部分に直接縫合して、閉鎖を試みています。しかしながら、呼吸運動によって常に動く臓器に対して、物理的な密閉を維持し続けることは技術的に困難であり、確実な修復法は依然として確立されていないのが現状です。このような背景から、高い閉鎖性能と安全性を兼ね備えた新たな医療機器の開発が強く望まれております。

現在主流となっている瘻孔閉鎖法には、ヒト血液由来の特定生物由来製品を接着剤として用いる方法がありますが、難治性瘻孔に対しては性能面で不十分な点が指摘されています。この課題をなんとか克服したいと長年考えていたところ、製剤学研究室の田原耕平教授と出会い、ナノファイバーシートという素材に触れる機会を得ました。生体内で使用される組織補強材には、柔軟性・粘着性・耐久性のいずれにも優れ、安全性が確保されていることが求められます。ナノファイバーが持つ卓越した物理特性は、これらの要件をバランス良く満たす可能性を秘めており、さらに生体適合性の高い合成高分子を基材として選択することで、安全性の面でも十分に対応可能です。

2023 年の冬より共同研究を開始し、田原耕平教授、三菱ケミカル株式会社 原幸嗣様、愛知医科大学 内藤宗和教授と一丸となり、ナノファイバーシートの改良を進めてまいりました。まだ課題は残されておりますが、約 1 年間の研究により、組織補強材としての性能は着実に向上しています。昨年度に大型動物モデルを用いた試験では、肺損傷部位を本シートで治療することに成功しました。この成果が認められ、令和 7 年度 医療機器等研究成果展開事業 チャレンジタイプに本研究課題が採択されました。

本シートは仕様の最適化によって、肺のみならず、肝臓・腎臓・腸管などの腹部臓器にも応用が期待されます。将来的には、抗炎症薬や成長因子などを組み込むことで、新たなドラッグデリバリーシステムとしての展開も視野に入れております。今後も、本開発物が臨床ニーズを満たす新たな医療機器として社会に貢献できるよう尽力してまいります。

最後になりましたが、本研究の推進にあたり多大なるご協力とご助言を賜りました、田原耕平教授、原幸嗣様、内藤宗和教授をはじめとする関係各位に、心より御礼申し上げます。今後とも変わらぬご指導・ご支援のほど、何卒よろしくお願い申し上げます。

Tomohito Okubo, MD

Assistant Professor
Aichi Medical University

As a thoracic surgeon, I specialize in the surgical treatment of lung cancer, intrathoracic infections, and cystic lung diseases. In my clinical practice, many patients present with compromised lung tissue due to factors such as smoking, and I often encounter challenging cases in which bronchopleural or alveolopleural fistulas fail to heal adequately.

When fistulas develop in air-containing organs such as the lungs or bronchi, the airflow passing through the fistulous tract during respiration interferes with wound healing. Even minute, pinhole-sized openings can require significant time to close. Consequently, secondary complications, such as infections, may arise, prolonging hospitalization and increasing physical and emotional burdens on both patients and healthcare providers.

Currently, when resection of the affected organ is not feasible, we attempt fistula closure by directly suturing autologous tissues—such as well-perfused fat or muscle—over the site. However, the constant movement of these organs due to respiratory dynamics makes it technically difficult to maintain a durable physical seal. To date, no universally reliable repair technique exists. This underscores the urgent need to develop novel medical devices that combine high sealing performance with safety.

The currently prevailing method for fistula closure involves the use of specific biologically derived adhesives made from human blood components. However, these adhesives have shown limited efficacy in treating intractable fistulas. While seeking a solution to this long-standing clinical challenge, I was opportune to meet Professor Kohei Tahara from the Department of Pharmaceutics, who introduced me to nanofiber sheets as potential materials. Tissue reinforcement materials intended for internal use must strike an optimal balance among flexibility, adhesiveness, and durability, while also demonstrating biocompatibility and safety. Nanofiber technology, which has excellent physical properties, holds great promise in meeting these requirements. By selecting biocompatible synthetic polymers as the base material, the safety profile of the sheets can be further enhanced.

Since the winter of 2023, we have been conducting joint research with Professor Kohei Tahara, Mr. Koji Hara of the Mitsubishi Chemical Corporation, and Professor Munekazu Naito of Aichi Medical University. Together, we have made continuous improvements to the nanofiber sheet. Although some challenges remain, we have significantly enhanced its performance as a tissue reinforcement material over the past year. In a preclinical study using a large animal model conducted last fiscal year, we successfully treated lung injuries using our nanofiber sheet. Consequently, our research project was selected for the AMED Medical Device Development Project (Challenge Type) for the current fiscal year.

With further optimization, this nanofiber sheet may find broader applications beyond the lungs—including liver, kidneys, and intestines. Going forward, we plan to incorporate anti-inflammatory drugs and growth factors into the material, aiming to develop a novel drug delivery system. We remain committed to advancing this technology with the hope that it will become a medical device capable of meeting unmet clinical needs and contributing meaningfully to patient care.

Finally, I would like to express my heartfelt gratitude to Professor Kohei Tahara, Mr. Koji Hara, Professor Munekazu Naito, and all others involved in this research for their invaluable guidance and support. I look forward to your continued collaboration and encouragement in the future.

Foreword
巻頭言

岐阜薬科大学 × MITSUBISHI CHEMICAL GROUP 三菱ケミカル株式会社

三菱ケミカル株式会社
スペシャリティマテリアルズビジネスグループ
ライフソリューションズ本部
フード・ヘルスケアインキュベーション部
新規事業推進グループ
マネジャー

原　幸嗣
Hara Koji

2022 年からはじまった寄附講座ですが、早くも3年が経過しました。その間の自主研究あるいは共同研究を通して多くの技術成果が得られたことについて、講座運営に携わっている方々、あるいはともに共同研究を進めて頂いた皆様方に、厚く御礼申し上げます。

化学会社である当社の高分子技術・素材と、岐阜薬科大学が保有する製剤技術・知見、加えて共同研究相手様の用途技術・情報をうまく融合活用することで、それぞれ単独で単独では成しえなかった研究成果をきわめて短期間で得ることができました。

寄附講座を設立していただいた背景の一つはオープンイノベーションによる研究開発の加速でした。実際に、研究を進めていく中で、当社製品はもちろんの事、他社製品をうまく活用することで、従来にない機能を付与したナノファイバーの開発に成功しています。

また、国内外の素材メーカーの皆様、ナノファイバー装置メーカーの方々、量産設備をお持ちのメーカー様等、ナノファイバーをキーワードとしてネットワークが大きく広がりました。

得られた成果をどのように社会実装していくかが次の課題と考えていましたが、これらの成果とネットワークを活用したスタートアップを、大学側ご支援の元、大学発ベンチャーとして、設立していただくことになりました。私も参画させていただく予定です。

寄附講座に加え、スタートアップの経営を通して、ナノファイバーを活用した創剤の社会実装を進めてまいります。引き続きのご支援をよろしくお願いいたします。

Koji Hara
Manager
Mitsubishi Chemical Corporation

Three years have passed since the establishment of the Endowed Course in 2022. I would like to express my sincere gratitude to everyone involved in its operation, as well as to our joint research partners, for the many technological advancements achieved through both independent and collaborative efforts—including the filing of almost ten patent applications.

By effectively integrating our polymer technologies and materials as a chemical manufacturer with the formulation expertise of Gifu Pharmaceutical University—and further incorporating the application technologies and insights of our joint research partners—we have achieved results that would have been difficult to realize independently, all within a remarkably short period.

A key motivation behind establishing the Endowed Course was to accelerate research and development through open innovation. During the course of our work, we successfully developed nanofibers with novel functionalities by skillfully utilizing our own products and those of other companies.

Furthermore, our nanofiber-focused collaborations have enabled us to significantly expand our network to include domestic and international materials manufacturers, nanofiber equipment manufacturers, and companies with mass production capabilities.

As we considered how best to translate these achievements into societal benefits, and with the support of the university, we decided to establish a university-launched startup that will leverage both the results and collaborative networks built through the Endowed Course. I also plan to participate in this venture.

Through both the Endowed Course and this startup initiative, we will continue working toward the social implementation of innovative nanofiber-based formulations. We sincerely appreciate your continued support.

Foreword
巻頭言

岐阜薬科大学
製剤学研究室
教授

田原　耕平
Tahara Kohei

岐阜薬科大学ナノファイバー創剤学寄附講座の2023年度および2024年度における活動をまとめた報告書を、こうして皆様にお届けできることを大変うれしく思います。本講座の運営にご協力いただいたすべての関係者の皆様に、心より感謝申し上げます。

本寄附講座は、2022年4月に三菱ケミカル株式会社（旧 日本合成化学工業株式会社）との連携により設置され、ナノファイバーという新たな製剤技術の社会実装を目指して活動を続けてまいりました。この2年間で、医薬品、化粧品、食品といった幅広い分野への応用可能性を探りながら、複数の企業・大学との共同研究が活発に進められ、学術的にも実用的にも大きな成果を挙げることができました。

本講座は、特任准教授としてご参画いただいている原幸嗣先生の専門的な知見とネットワークを軸に、現場のニーズと研究の橋渡しを意識した活動を展開しています。現場で求められる「確実で再現性のある製造技術」と、大学が持つ「新規性のある基礎研究」とを結びつけることは容易ではありません。しかし、本講座では、オープンイノベーションの考え方を重視し、産官学の壁を越えた連携によって、着実に研究成果を社会に届けるための礎を築いてきました。

また、近年では医薬品開発における製剤化工程（ダウンストリーム）において、ナノファイバー技術の有効性が徐々に注目されるようになっています。従来技術に比べて導入のハードルが高いとされる中で、科学的エビデンスの積み重ねと、粘り強い実証研究が本講座の信念であり、強みでもあります。

そして、ここでご報告すべき重要な出来事として、2025年4月1日より岐阜薬科大学は、従来の岐阜市の直営から「岐阜市公立大学法人」として新たな一歩を踏み出しました。公立大学法人化により、大学運営の柔軟性と意思決定の迅速化が可能となり、研究成果の社会実装や大学発ベンチャーの創出に向けた環境が大きく前進しています。

こうした大学の制度的な変革の波を受け、本寄附講座で蓄積してきたナノファイバー創剤の研究成果を、より実践的かつ持続的な形で社会に展開すべく、2025年度中に大学発スタートアップ企業「ジェノフィブリクス（Genofibrix）」の設立を計画しております。このスタートアップでは、医薬品はもちろん、化粧品や機能性食品への応用も視野に入れ、研究成果を具体的な製品・技術として世に送り出すことを目指します。

新しい制度のもと、大学とスタートアップが連携しながら研究と事業を推進していくことで、ナノファイバー技術の価値を最大限に引き出し、社会課題の解決にも貢献できるものと確信しております。本報告書が、本講座の取り組みとその将来展望を共有する一助となり、新たな共創のきっかけになれば幸いです。

今後とも、変わらぬご指導とご支援を賜りますよう、何卒よろしくお願い申し上げます。

Kohei Tahara, Ph.D.

Professor, Laboratory of Pharmaceutics
Gifu Pharmaceutical University

It is with great pleasure that I present this report summarizing the activities of the Gifu Pharmaceutical University Endowed Chair for Nanofiber-Based Drug Formulation Science for the 2023 and 2024 academic years. I would like to express my sincere gratitude to everyone who has supported the operation of this endowed chair over the past two years.

Established in April 2022 in collaboration with Mitsubishi Chemical Corporation (formerly Nippon Synthetic Chemical Industry Co., Ltd.), this endowed chair is dedicated to advancing the societal implementation of nanofiber technologies as novel pharmaceutical formulations. Over the past two years, we have steadily pursued academic and industrial progress by launching collaborative research projects with various companies and universities across the fields of pharmaceuticals, cosmetics, and functional foods.

Under the leadership of Specially Appointed Associate Professor Dr. Koji Hara—who brings deep expertise in polymers and pharmaceuticals, along with an extensive collaborative network—we have made significant strides in linking practical pharmaceutical manufacturing needs with fundamental academic research. Our aim is to bridge this gap through open innovation and translational science.

In pharmaceutical development, drug formulation is typically considered a downstream process. once drug candidates are identified, there is often a demand for rapid and reliable formulation. However, because formulation must ensure both product quality and manufacturing stability, new technologies are often introduced with caution. At our chair, we address this challenge by incorporating practical manufacturing insights from the onset and building a solid scientific foundation for the effectiveness of nanofiber formulations—facilitating their early adoption in real-world settings.

One major institutional milestone occurred on April 1, 2025: Gifu Pharmaceutical University transitioned from being directly operated by Gifu City to becoming part of the newly established Gifu City Public University Corporation. This shift to a public university corporation has enabled more agile decision-making and flexible university management, fostering an environment more conducive to securing research funding and launching university-based startups.

Considering this transformation, we are currently planning to establish a university spinoff—Genofibrix—in fiscal year 2025, with the goal of translating our research outcomes into innovations with real-world impact. Genofibrix will leverage nanofiber formulation technologies not only for pharmaceuticals but also for cosmetics and functional foods, delivering advanced materials and products that address diverse societal needs.

By aligning our research initiatives with this new institutional framework and strengthening collaboration between the university and our startup, we are confident in our ability to maximize the potential of nanofiber technology and contribute meaningfully to addressing societal challenges.

We hope that this report offers valuable insights into our ongoing activities and future direction and serves as a springboard for further collaborative initiatives. We greatly appreciate your continued support and guidance.

Members & Research Themes
メンバー・研究テーマ

ナノファイバー創剤学寄附講座
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吉安 有見子
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大学院生
Graduate Student

アラッシュ　ヤブアリ
Arash Yavari

研究補助員
Research Assistants

小出 陽子
Yoko Koide

山田 真弓
Mayumi Yamada

研究目標
Research Objectives

近年、医薬品分野では抗体医薬品や新型コロナウイルスワクチンに代表される RNA 医薬品や遺伝子治療など治療手段（モダリティ）が多様化しています。特に新規医薬品は安定性や吸収性に問題を抱えていることが多いため、効果を最大限に発揮できる製剤化技術が強く望まれています。このような背景の中、電界紡糸（エレクトロスピニング）法により調製される高分子ナノファイバー（ナノ繊維）は、医薬品製剤化プラットフォームになると期待されています。高分子ナノファイバーは、経口製剤（錠剤やフィルム製剤など）、外用剤（貼付剤）、舌下フィルムなど粘膜適用製剤、吸入剤などに様々な剤形に応用することが可能です。

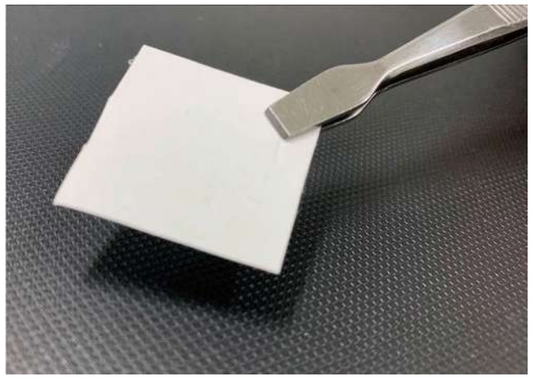
エレクトロスピニング法は熱を使わないマイルドな条件下で液体原料を直接固形化することができます。本講座では医薬品だけでなく、不安定な化合物を扱うことが多い機能性食品や化粧品分野にも積極的な応用展開を行っています。岐阜薬科大学製剤学研究室と連携してメディカルナノファイバーを新剤形として開発（創剤）し、研究成果の社会実装が本寄附講座の目標です。また、薬学と工学の両方を理解し医薬品開発に貢献できる人材育成を目指します。

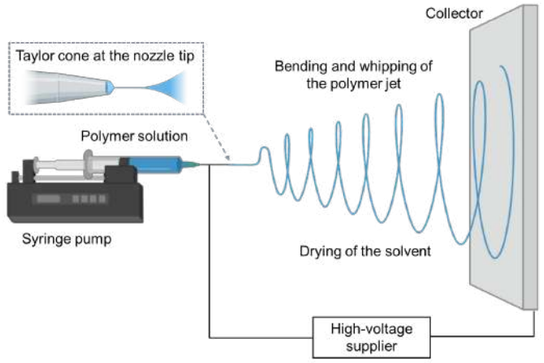
In recent years, the pharmaceutical field has seen diversification of therapeutic modalities, such as antibodies, RNA medicines (typified by novel coronavirus vaccines), and gene therapy. Innovative drugs often have stability and absorption, issues that have led to increased interest in developing formulation technologies that can maximize the therapeutic effects of these drugs. Given this background, polymeric nanofibers (nanofibers) prepared by field spinning (electrospinning) are expected to become a platform for pharmaceutical formulation development. Polymeric nanofibers can be applied to various dosage forms, such as oral (e.g., tablets and film formulations), topical (transdermal patch, sublingual patch, and other formulations for mucosal tissues), and inhalation products.

The electrospinning method can directly formulate liquid raw materials into solids under mild conditions without the use of heat. In this laboratory, we are actively developing applications not only for pharmaceuticals, but also for functional foods and cosmetics, in
which unstable compounds are often used. Medical nanofibers will be developed as a new dosage form in collaboration with the Laboratory of Pharmaceutical Engineering (Tahara research group) at Gifu Pharmaceutical University. We are also actively engaging in joint research with research institutes, industries, and hospitals to translate research data on the use of medical nanofibers into practical applications.

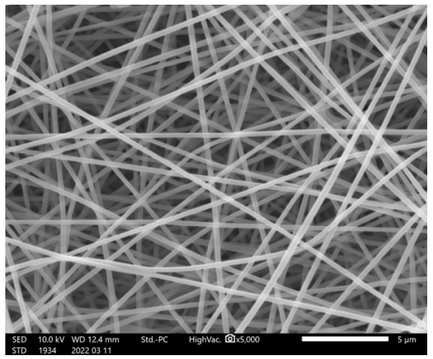
Members & Research Themes

メンバー・研究テーマ

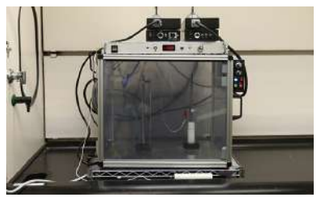


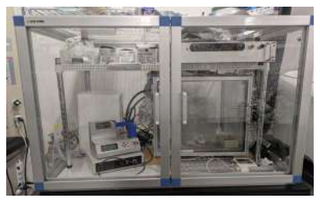


Preparation of nanofibers by electrospinning method



SEM image of polymeric nanofibers





研究テーマ

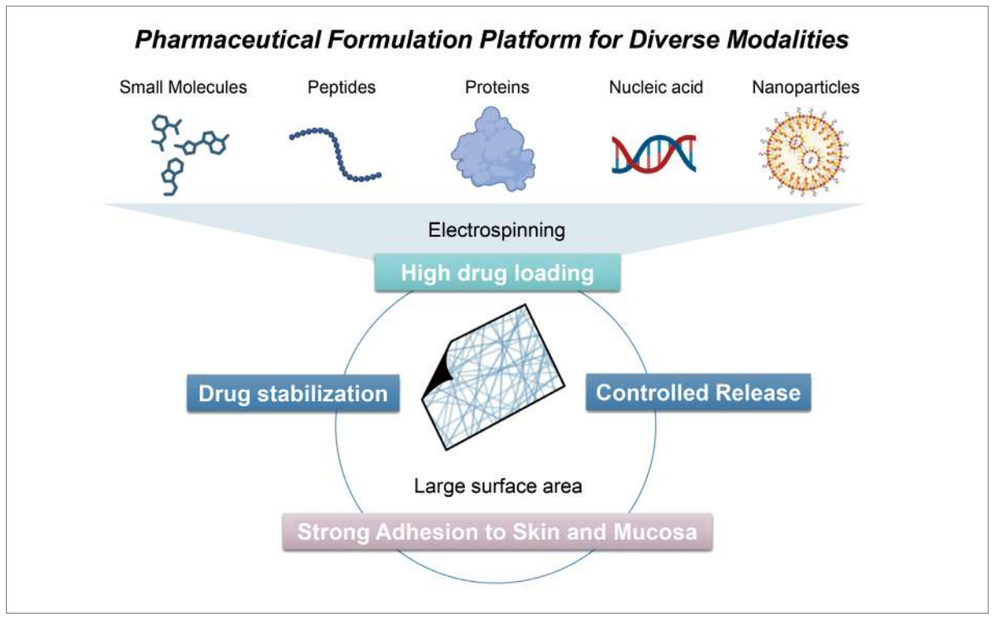
Research Topics

1. ナノファイバー基剤の探索と開発
   Discovery and development of polymeric materials for nanofiber substrates

2. 難水溶性薬物の溶解性改善を目指したナノファイバー固体分散体の開発
   Development of solid-dispersion nanofibers to improve the solubility of poorly water-soluble drugs

3. バイオ医薬品・核酸医薬品など新しいモダリティに対する製剤化技術としてのナノファイバー応用
   Nanofiber application as a formulation technology for new modalities such as biopharmaceuticals and nucleic acid medicines

4. ナノファイバーを活用する経粘膜・経皮など低侵襲 DDS 製剤の開発
   Development of noninvasive DDS formulations utilizing nanofibers, such as transmucosal and transdermal administrations



Achievements
研究業績

1. 論文 Papers

Iwai M, Yamazoe E, Ito T, Hara K, Tahara K. Design of an oral formulation combining PVA nanofibers and PEGylated liposomes for enhanced drug delivery. *J. Drug Deliv. Sci. Technol*. 101, 106285. 2024

Miki A, Hara K, Shibata T, Morioka T, Kobayashi A, Yoshimura N, Yamazoe E, Ito T, Tahara K. Development of a solid dispersion system for polyvinyl alcohol nanofibers embedded with silicon dioxide particles via emulsion electrospinning for improved solubility of poorly water-soluble drugs. *J Drug Deliv Sci Technol*. 99: 105915. 2024

Ito T, Tamashiro S, Okuda H, Yamazoe E, Tahara K. Cryomilled electrospun nanofiber mats containing d-mannitol exhibit suitable for aerosol delivery of proteins. *Int J Pharm*. 661: 124425. 2024.

Kanamori M, Hara K, Yamazoe E, Ito T, Tahara K. Development of Polyvinyl Alcohol (PVA) Nanofibers Containing Cationic Lipid/siRNA Complexes via Electrospinning: The Impact of PVA Characterization. *Nanomaterials*. 14: 1083. 2024.

Ogawa R, Hara K, Kobayashi A, Yoshimura N, Taniguchi Y, Yamazoe E, Ito T, Tahara K. Controlled Release of Lysozyme Using Polyvinyl Alcohol-Based Polymeric Nanofibers Generated by Electrospinning. *Chem. Pharm. Bull*. 72: 324-329. 2024.

田原耕平 , 原幸嗣
エレクトロスピニング法を用いたナノファイバーの製剤応用と薬物封入技術
製剤機械技術学会誌 Vol.33 No.5 27-34. 2024

原 幸嗣, 田原耕平
エレクトロスピニング法によるナノファイバーの医薬品応用
PHARM TECH JAPAN Vol.39 No.8 171-177. 2023

2. 特許出願 Patents

- 特願 2025-37776　損傷部位治療用ナノファイバーシート、その製造方法及びその使用方法
- 特願 2024-59421　眼球貼付剤
- 特願 2024-48905　脂質ナノ粒子含有ナノファイバー、及びその製造方法
- 特願 2022-133803　薬物含有ファイバー、およびそのファイバーからなる経口製剤
- 特願 2022-012541　ポリビニルアルコール系樹脂含有ナノファイバー
- 特願 2022-162638　固体分散体用基剤

3. 学士論文 Undergraduate Thesis

- 荒川　咲紀　難水溶性薬物の溶解性制御を目的とした高分子ナノファイバー固体分散体に関する研究
- 玉城　慎太郎　粉砕補助剤として糖類を添加したエレクトロスピニング法吸入粉末剤の開発
- 宇田　翔夢　持続的薬物送達可能な眼疾患用ナノファイバーパッチの設計
- 奥田　寛生　吸入粉末剤調製を志向した電界紡糸ナノファイバーマットの調製・粉砕条件の検討
- 金森　美有　エレクトロスピニング法による siRNA 含有ナノファイバーの設計
- 和田　桃佳　抗老化候補物質 NMN の経口製剤設計に関する研究

4. 学内講演会 On-Campus Seminar

- **第 2 回ナノファイバー創剤学寄附講座主催セミナー**
  2023 年 9 月 22 日（金）10:00-11:00
  岐阜薬科大学・本部キャンパス 2 階 第 2 講義室
  講師：野本 貴大 先生（東京大学大学院総合文化研究科 准教授（PI））
  講演タイトル：「光線力学療法・ホウ素中性子捕捉療法の適応拡大を目指したドラッグデリバリーシステムの開発」

Achievements
研究業績

5. 学会発表 Conference Presentations

【2024 年】

・商工課マッチングツアー
2024 年 12 月 18 日・岐阜
高分子ナノファイバーを活用した創剤・DDS 開発

・第 1 回 C-DAM カンファレンス
2024 年 12 月 10 日・名古屋
高分子ナノファイバーを活用した創剤・DDS 開発

・第 41 回製剤と粒子設計シンポジウム
2024 年 11 月 12 日・岡山
電界紡糸ナノファイバーを用いた固形製剤設計と薬物放出制御

・日本病院薬剤師会東海ブロック・日本薬学会東海支部合同学術大会 2024
2024 年 10 月 27 日・岐阜
電界紡糸技術を用いた持続的薬物送達可能な眼疾患用ナノファイバーパッチの設計

・2024 AAPS PharmSci 360
2024 年 10 月 22 日・Salt Lake City, USA
Development of inhaled dry powder using electrospinning method

・第 29 回創剤フォーラム 若手研究会
2024 年 9 月 12 日・静岡
エレクトロスピニング法を用いた薬物持続放出型眼疾患用製剤の開発

・粉体工学会 中部談話会　省エネルギーに貢献する粒子設計・粉体プロセスの薬工連携研究会
2024 年 9 月 5 日・静岡
エレクトロスピニング技術を用いた眼疾患用パッチ製剤の開発

・iNexS マッチング会
2024 年 9 月 25 日・湘南アイパーク
高分子ナノファイバーを活用した創剤・DDS 開発

・第 70 回 日本薬学会 東海支部総会・大会
2024 年 7 月 6 日・名古屋
エレクトロスピニング法吸入粉末剤の調製における粉砕補助剤の影響

・CRS 2024 Annual Meeting and Expo
July 8-12, 2024・Bologna, Italy
Controlled release of lysozyme using polyvinyl alcohol-based polymeric nanofibers generated by electrospinning

・CRS 2024 Annual Meeting and Expo
July 8-12, 2024・Bologna, Italy
PVA nanofibers with PEGylated liposomes for small intestinal mucosal delivery

・ファーマラボ EXPO [東京] アカデミックフォーラム
2024 年 6 月 27 日・東京ビッグサイト
高分子ナノファイバーを活用した創剤・DDS 開発

・日本薬剤学会 第 39 年会
2024 年 5 月 25 日・神戸
エレクトロスピニング (電界紡糸) 技術を用いた吸入剤開発

・日本薬剤学会 第 39 年会
2024 年 5 月 25 日・神戸
エレクトロスピニング (電界紡糸) 法を用いた naked plasmid DNA 吸入粉末剤の開発

・2024 年度 C-DAM 第 1 回シーズ・情報共有部会
2024 年 5 月 14 日・オンライン
エレクトロスピニングによるナノファイバーの医薬品・医療機器への応用

・日本薬学会第 144 年会（横浜）
2024 年 3 月 28 日・横浜
難水溶性薬物の溶解性制御を目的とした高分子ナノファイバー固体分散体の設計

【2023 年】

・第 40 回製剤と粒子設計シンポジウム
2023 年 11 月 20 日・姫路
消化管粘膜滞留性と透過性の向上を目的としたリポソーム含有ナノファイバー製剤の設計

Achievements
研究業績

・情報機構オンラインセミナー
2023 年 9 月 25 日・オンライン
エレクトロスピニングによるナノファイバーの医薬品・医療機器への応用〜最近の動向と今後の展望〜

・粉体工学会　省エネルギーに貢献する粒子設計・粉体プロセスの薬工連携研究会
2023 年度若手研究者討論会　2023 年 9 月 20 日・愛知
粉砕補助剤を添加したエレクトロスピニング法吸入粉末剤の調製

・第 2 回東海地区創薬デザイン研究会
2023 年 8 月 28 日・岐阜
Development of Novel Nanofiber Wound Dressings: Design and Process Optimization of Polyamide-6 Nanofibers by Electrospinning

・日本薬剤学会 第 38 年会　学術シンポジウム 5
2023 年 5 月 18 日・名古屋
Additive manufacturing と Electrospinning による製剤設計の可能性

・日本薬剤学会 第 38 年会
2023 年 5 月 16 日・名古屋
高分子薬物の経口製剤化を目的としたリポソーム含有ナノファイバー製剤の設計

6. その他 Others

・インタビュー　エレクトロスピニング法を用いたナノ繊維による創剤技術を開発
田原耕平 , 原幸嗣
Nonwovens review Vol.33 No.4 p.2-6 (2023)

Newspaper Article

三菱ケミカルが1500万円

紺綬褒章 岐阜薬科大講座に寄付

三菱ケミカルは、岐阜薬科大のナノファイバー創剤学寄付講座に1500万円を寄付した。公益のため寄付をした功績で、同社に贈られた紺綬褒章の伝達式が、岐阜市役所で行われた。

褒状を受ける楠本医ライフソリューションズ本部長（左）＝岐阜市役所

講座は、医薬品の製剤化に役立つことが期待されるナノファイバーの研究で、薬学と工学を連携した新技術開発などを目指し、昨年4月に設置された。

伝達式では、同大を運営する同市の後藤一郎副市長から、同社スペシャリティマテリアルズビジネスグループの楠本医ライフソリューションズ本部長に褒状が手渡された。楠本本部長は「ユニークな取り組みで大学と事業者とが一緒により高い価値を生み出すことを期待している」と語った。（小田香緒里）

・新聞記事　2023年11月17日　岐阜新聞
紺綬褒章 岐阜薬科大講座に寄付

薬学とSDGsの融合

常温で製剤 省エネに

極薄シートで新薬へ道

田原耕平教授

・新聞記事　2023年11月24日　中日新聞　岐阜薬科大特集記事
薬学と SDGs の融合（常温で製剤　省エネに）

nanomaterials

MDPI

Article

Development of Polyvinyl Alcohol (PVA) Nanofibers Containing Cationic Lipid/siRNA Complexes via Electrospinning: The Impact of PVA Characterization

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Abstract: This study aimed to develop polyvinyl alcohol (PVA) nanofibers encapsulating 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP)/siRNA complexes via electrospinning for the delivery of nucleic acid-based drugs. It also focused on the influence of the intrinsic properties of PVA on the efficacy of the system. PVA nanofibers, with diameters of 300–400 nm, were obtained, within which the siRNA remained intact and the DOTAP/siRNA complexes were uniformly dispersed. By incorporating DOTAP/siRNA complexes into the PVA nanofibers and assessing the impact of their RNA interference (RNAi) activity in A549-Luc cells, a stable inhibition of luciferase expression was observed. An examination of the nanofiber preparation process revealed that even when DOTAP or siRNA were added separately to the PVA solution without forming complexes, the RNAi effect was retained. The DOTAP/siRNA complexes released from the PVA nanofibers were internalized by the cells, with some PVA residues remaining on their surfaces. The significance of the degree of hydrolysis and polymerization of PVA on the performance of nanofibers was highlighted. Notably, PVA with a low degree of hydrolysis substantially enhanced RNAi effects, with luciferase expression inhibition reaching 91.5 ± 0.7%. Nanofibers made of PVA grades with anionic or cationic modifications were also evaluated, suggesting that they affect the efficacy of siRNA delivery. The insights obtained suggest avenues for future research to optimize drug delivery systems further.

Keywords: polyvinyl alcohol; nanofibers; electrospinning; siRNA; cationic lipid

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1. Introduction

Electrospinning is a technique employed for fabricating nanofibers by applying high-voltage electrostatic forces to a polymer solution within a needle-like nozzle. The physical properties of the nanofibers can be adjusted by altering the polymer, solvent, and parameters such as solution flow rate, as well as applied voltage. Electrospinning is notable for its rapid solvent evaporation owing to the high surface area of the nanofibers, which enables manufacturing at room temperature. This ability makes it a promising technique for applications in tissue engineering, regenerative medicine, preparing wound dressing materials, and synthesizing pharmaceutical formulations [1]. Generally, electrospinning involves applying high voltages ranging from 10 to 40 kV to the solution, but with a very low current in the microampere range; thus, energy consumption is also low. This process, conducted at room temperature, is suitable for unstable biomolecules and advantageous for nucleic acid-based pharmaceuticals despite challenges in productivity [2].

Incorporating nucleic acids, such as siRNA and mRNA, into nanofiber scaffolds to imitate the extracellular matrix is crucial for tissue and stem cell engineering [3–7]. siRNAs encapsulated within nanofiber scaffolds function as controlled-release reservoirs, providing potentially long-term gene therapeutic effects as well as guiding and supporting seeded cells [8,9]. For this purpose, hydrophobic polymers, which are less soluble in cell culture media, such as polycaprolactone or poly(lactide-co-glycolide), are often used as matrices [10].

Polymer nanofibers produced by electrospinning are suitable for transdermal treatment systems and fabricating wound dressing materials [11,12]. Moreover, there have been numerous reports on their applicability in site-specific drug release in the gastrointestinal tract via oral administration, mucosal vaccines for sublingual application, and localized drug therapy in mucosal tissues such as the eyes, lungs, and vagina [13–18]. The development of nucleic acid pharmaceuticals incorporated into these polymer matrix nanofibers is anticipated. Electrospinning, as a room-temperature process, is a promising alternative to freeze-drying for solidifying nucleic acid pharmaceuticals. In cases where nanofibers are directly applied to patients, polymers with high water solubility that eventually dissolve or degrade are more likely to be selected.

This study used water-soluble polyvinyl alcohol (PVA), a prevalent excipient in pharmaceutical formulations, as a foundational matrix for nanofiber-encapsulating siRNAs, a category of nucleic acid therapeutics. Recent reports have demonstrated the virtue of PVA nanofibers in drug delivery applications, highlighting their potential for controlled release and biocompatibility [19–21].

The physical characteristics of PVA, which predominantly include its water solubility, are determined by its degree of hydrolysis (ratio of hydroxyl to acetyl groups) and polymerization (number of monomers) [22]. The selection of PVA grades allows for tailored water affinity, rendering PVA nanofibers potent candidates for the controlled release of nucleic acid drugs. Through cross-linking, PVA can be rendered insoluble, a property leveraged in preparing wound dressing materials. Additionally, materials that are not inherently suitable for electrospinning into nanofibers, such as carboxymethyl cellulose, can be engineered into nanofibers via hybridization with PVA [23]. Numerous applications for hybrid nanofibers that combine PVA with various functional polymers have been documented [24]. For instance, gellan/PVA nanofibers exhibiting mucosal adhesion and gastric retention [25], gelatin/PVA nanofibers optimized for wound healing [26], and chitosan/PVA and polyvinylpyrrolidone/PVA nanofibers for transdermal drug delivery systems have been developed [27,28]. Consequently, a comprehensive analysis focusing on the encapsulation efficiency of nucleic acid drugs and their cellular uptake efficiency in relation to the different grades of PVA is imperative to optimally harness PVA-based nanofibers as a versatile platform for delivering nucleic acid drug formulation.

In nanofiber-based formulations of nucleic acid drugs, the integration of cationic lipids or alternative transfection agents is essential. Typically, positively charged complexes formed with nucleic acid drugs engage in electrostatic interactions with negatively charged cell membranes, facilitating their adsorption. Subsequently, these complexes are internalized by the cells via endocytosis, which is critical for the functioning of the drugs.

This study aimed to fabricate siRNA-laden nanofibers by electrospinning, and siRNA was employed as a nucleic acid-based therapeutic agent. For siRNA transfection, 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), a cationic lipid with a quaternary ammonium group, was chosen [29]. Our approach involved embedding the DOTAP/siRNA complex within PVA nanofibers through electrospinning. PVA with a hydrolysis degree of 88% and high polymerization (GOHSENOL™ EG-40P), commonly used as a pharmaceutical excipient, served as the standard. We first prepared DOTAP/siRNA-encapsulated PVA nanofibers using EG-40P to optimize the preparation methods. The different preparation methodologies on the intracellular distribution and RNA interference (RNAi) efficacy of the siRNA was assessed using EG-40P nanofibers. This evaluation was conducted on A549-Luc cells, which are derived from human non-small cell lung cancer and exhibit stable luciferase expression. Additionally, the influence of varying PVA grades, distinguished by

their degree of polymerization and saponification, on the inhibition of luciferase activity by siRNA was explored.

2. Materials and Methods

2.1. Materials

PVAs (GOHSENOL™ EG-40P, EG-05P, EG-30P, KH-20, NH-18, GOHSENX™ K-434, and T-330) were provided by the Mitsubishi Chemical Corporation (Tokyo, Japan). Table 1 presents the degrees of polymerization and hydrolysis of PVAs used in this study. PVAs are abbreviated according to their degrees of polymerization and hydrolysis; for example, PVA24-88 has a 24 × 10^2 degree of polymerization and an 88% degree of hydrolysis. Annealed siRNA targeting pGL3 firefly luciferase (Luc-siRNA, sense: 5′-CUUACGCUGAGUACUUCGAdTdT-3′, and antisense: 3′-dTdTGAAUGCGACUCAUGAAGCU-5′) was purchased from Nippon Gene (Tokyo, Japan). DOTAP (cationic lipid) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). The Hoechst 33342 stain was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). All other chemicals used were of the highest commercially available grade.

Table 1. Characteristics of different PVA grades and the respective PVA concentrations for electrospinning used in this study.

Characterization of PVA					PVA Concentration in Solution(%) Used for Electrospinning
Abbreviation	**PVA Grade**	**Degree of Polymerization**	**Degree of Hydrolysis (mol %)**	**Note**	
PVA24-88	GOHSENOL™EG-40P	2400	88		8
PVA20-88	GOHSENOL™EG-30P	2000	88		8
PVA6-88	GOHSENOL™EG-05P	600	88		20
PVA25-80	GOHSENOL™KH-20	2500	80		8
PVA17-98	GOHSENOL™NH-18	1700	98		8
Cationic PVA	GOHSENX™ K-434	1500	88	Modified PVA having a cationic group (quaternary ammonium salt)	8
Anionic PVA	GOHSENX™ T-330	1900	95	Modified PVA having a carboxyl group	8

2.2. Preparation of DOTAP/siRNA Complexes

To prepare DOTAP/sRNA complexes with a nitrogen-to-phosphorus (N–P) ratio of 5, 10, or 100 µL of 20 µM siRNA solution in Milli-Q water was added to an equal volume of DOTAP suspension in Milli-Q water (the final volume was 200 µL, and the final concentration of siRNA was 10 µM).

2.3. Preparation of siRNA-Loaded PVA Nanofibers by Electrospinning

In Method A, 25 g of 8% or 20% (*w*/*w*) PVA aqueous solution was prepared, to which 200 µL of either siRNA solution or DOTAP/siRNA complex (10 µM siRNA) was added. As an alternative approach, in Method B, DOTAP or siRNA were added separately to the PVA aqueous solution to prepare a precursor for electrospinning. To incorporate fluorescein isothiocyanate-labeled PVA (FITC-PVA), prepared as per a previously reported method [30], into the nanofibers, a PVA solution with a ratio of unlabeled PVA to FITC-PVA at 1:9 (*w*/*w*) was employed. This mixture was then loaded into a 1 mL syringe connected via silicone tubing to a 22G non-beveled needle with an inner diameter of 0.4 mm (Terumo, Tokyo, Japan). Electrospinning of the nanofibers onto a plate-type collector was facilitated by a high voltage of 10 kV from an HVU-30P100 high-voltage power supply (MECC Co., Ltd., Fukuoka, Japan) [22]. The distance between the needle and the collector was maintained at 12 cm, and the solution was delivered to the needle at a flow rate of 0.5 mL/h using a syringe pump (Yutaka Electronics Manufacturing, Gifu, Japan).

2.4. Physicochemical Properties of the siRNA-Loaded PVA Nanofibers

The particle size and zeta potential of the DOTAP/siRNA complexes, immediately after preparation and after release from the PVA nanofibers dissolved in Milli-Q water, were measured after appropriate dilution by employing a Zetasizer Nano ZS instrument (Malvern, Worcestershire, UK). The nanofiber mats prepared by electrospinning were imaged using a JSM-6510LV scanning electron microscope (SEM; JEOL, Tokyo, Japan). The mean and standard deviation of the fiber diameter were calculated by measuring 100 randomly selected points on SEM images using the ImageJ™ image analysis software, version 1.53, created by Wayne Rasband, National Institutes of Health, Bethesda, MD, USA, accessed on 20 May 2022, from https://imagej.net/ij/. The siRNA content of the recovered nanofibers dissolved in Milli-Q water was determined using high-performance liquid chromatography (HPLC). For siRNA detection, an EXTREMA HPLC System (JASCO, Tokyo, Japan) equipped with a Waters Xselect CSH C18 column (5 µm, 250 × 4.6 mm i.d.) was used. The detection wavelength was 260 nm. The mobile phase consisted of 0.1 M ammonium bicarbonate solution + acetonitrile in a ratio of 80:20 (*v*/*v*). The analysis was performed using an isocratic method with a sample injection volume of 10 µL, a solvent flow rate of 1 mL/min, and a column temperature of 30°C. The encapsulation efficiency (%) was ascertained by dividing the amount of siRNA detected using HPLC by the initial amount of siRNA added during the preparation of the nanofibers. PVA nanofibers loaded with FITC-PVA and carboxy tetramethyl rhodamine (TAMRA)-labeled siRNA were observed using an LSM-700 confocal laser scanning microscopy (CLSM; Carl Zeiss, Oberkochen, Germany).

2.5. Cell Line and Culture

The A549-Luc cell line (#JCRB1414) was obtained from the JCRB Cell Bank (National Institute of Biomedical Innovation, Osaka, Japan). It was maintained in E-MEM (051-07615; Fujifilm Wako Pure Chemical) supplemented with 10% (*v*/*v*) fetal bovine serum (Lot No. 1638593; Sigma, Tokyo, Japan), 1% MEM non-essential amino acids (139-15651; Fujifilm Wako Pure Chemical), and 1% penicillin-streptomycin (15140122; Thermo Fisher Scientific, Waltham, MA, USA) at 37°C under 5% CO_2.,

2.6. Evaluation of the Luciferase-Knockdown Efficiency of siRNA in A549-Luc Cells

A549-Luc cells were seeded in a 24-well plate at a cell density of 2.5 × 10^4 cells/cm^2 and cultured for 24 h before treatment. A suspension of the DOTAP/siRNA complexes diluted in a serum-free medium was used for this evaluation. Additionally, all types of siRNA-containing PVA nanofibers were fully dissolved in the serum-free medium, releasing the DOTAP/siRNA complexes, and they were employed for the examination. In this experiment, the PVA nanofibers were dissolved for >30 min, and the absence of any PVA nanofiber remnants in the solution was confirmed before adding it to the cells. After washing the cells once with Hanks' balanced salt solution (HBSS), 0.5 mL of each sample was added to each well to achieve a final siRNA concentration of 100 nM. Then, they were incubated for 4 h in a CO_2 incubator. Next, the samples were removed, and the cells were washed with HBSS. Subsequently, the culture medium was added, and the cells were further cultured for 22 h in the CO_2 incubator. After this, the cells were lysed with a Reporter Lysis 5X Buffer (Promega, Madison, WI, USA) and collected by centrifuging the cell lysates at 18,000× *g* for 2 min and 4 °C. The luciferase activity in the collected supernatant was quantified by employing the PicaGene Luminescence Kit (Toyo Ink, Tokyo, Japan), followed by measuring the luminescence with a GloMax® 20/20 Luminometer (Promega, Tokyo, Japan). The protein concentration was determined using the BCA Protein

Assay (Pierce, Rockford, IL, USA), and the luciferase activity was converted to relative light units/mg of protein.

2.7. Intracellular Distribution of the siRNA in A549-Luc Cells Observed by CLSM

A549-Luc cells were seeded onto the Lab-Tek® II Chambered #1.5 German Coverglass System (Nalge Nunc International, Naperville, IL, USA) at a density of 2.5×10^4 cells/cm^2 and incubated for 24 h. Subsequently, the cells were washed with HBSS and treated with DOTAP/TAMRA-siRNA complexes (N/P ratio = 5) encapsulated within FITC-PVA (EG-40P) nanofibers, which were fully dissolved in a serum-free medium to a final siRNA concentration of 100 nM. Of this, 500 µL was added to each well. After incubation for 4 h in a CO_2 incubator, the siRNA samples were removed, and the cells were washed with HBSS. Cell nuclei were stained by incubation with 10 µg/mL Hoechst 33342 for 20 min. After washing with HBSS, the cells were fixed with 200 µL of 4% paraformaldehyde. The intracellular fluorescence distribution was ascertained using an LSM-700 CLSM.

2.8. Cytotoxicity Assays

A549-Luc cells were seeded in a 96-well plate at a density of 7.81×10^4 cells/cm^2 and incubated for 24 h. The cells were then washed with HBSS and treated with DOTAP/siRNA (N/P ratio = 5)-containing PVA nanofibers fully dissolved in a serum-free medium. The solution was adjusted to a final siRNA concentration of 100 nM by adding 0.1 mL to each well. Specifically, three types of PVA nanofibers synthesized from the PVA grades: EG-40P, K-440, and T-330, were evaluated. The cells were then incubated for 4 h in a CO_2 incubator. After another wash with HBSS, a mixture of cell culture medium and WST-8 reagent from the Cell Counting Kit-8 kit (Dojindo Laboratories, Kumamoto, Japan). was added to each well in a ratio of 10:1 (*v*/*v*). Following incubation for 30 min in the CO_2 incubator, OD_{450} was measured using a GloMax Multi Detection System microplate reader (Promega).

3. Results and Discussion

3.1. Characterization of DOTAP/siRNA-Containing PVA Nanofibers

Preliminary studies evaluated the particle size and zeta potential of the DOTAP/siRNA complexes prepared by varying the N/P ratio from 0.5 to 10. In general, positively charged nanoparticles interact strongly with the oppositely charged cell membranes and are readily internalized by the cells via endocytosis [29]. At an N/P ratio of 5, the complexes exhibited a high positive charge (30.1 mV), and therefore, they were used. Nanofibers electrospun solely from PVA (EG-40P) solution yielded relatively linear fibers with an average diameter of 473 ± 57 nm (Figure 1a). The siRNA encapsulation efficiency in PVA nanofibers, measured using HPLC, was 94.5 ± 1.5%. This elevated drug encapsulation efficiency in electrospun fibers could be attributed to the drug and polymer solubilities in water, thus minimizing drug loss during encapsulation [31,32]. Incorporation of either siRNA or DOTAP/siRNA complexes (N/P ratio = 5) slightly reduced the fiber diameters to 382 ± 43 nm and 311 ± 88 nm, respectively (Figure 1b,c), presumably because of the enhanced conductivity of the PVA solution [33].

CLSM observations were conducted on fibers containing FITC-PVA and TAMRA-siRNA to observe the distribution of lipoplexes within the nanofibers (Figure 1d). The punctate dispersion of TAMRA-siRNA fluorescence within the PVA nanofibers confirmed the uniform distribution of the DOTAP/siRNA complexes. Subsequently, the particle size distribution and zeta potential of DOTAP/siRNA-containing PVA nanofibers dissolved in Milli-Q water were compared with those of the freshly prepared complexes. This experiment ascertained whether DOTAP/siRNA was released from the PVA nanofibers. Figure 1e shows a comparison of the particle size distribution of the DOTAP/siRNA complexes immediately after preparation and release from PVA nanofibers, which were completely dissolved in water. Due to the simple mixing of each solution, the freshly prepared DOTAP/siRNA complexes exhibited multiple peaks in the submicron range, lacking precise particle size control. Although there was no exact match in the peaks of

the freshly prepared DOTAP/siRNA complexes and those released from the dissolved PVA nanofibers, the average particle size for both was <1 µm. This finding suggests the preservation of the integrity of the DOTAP/siRNA complexes within the nanofibers. However, the zeta potential of the freshly prepared complexes was 30.1 mV, while that of the DOTAP/siRNA complexes within the PVA nanofibers was reduced to 4.3 mV. This decline indicates that the residual PVA was adsorbed or remained on the surface of the DOTAP/siRNA complexes released from the nanofibers.

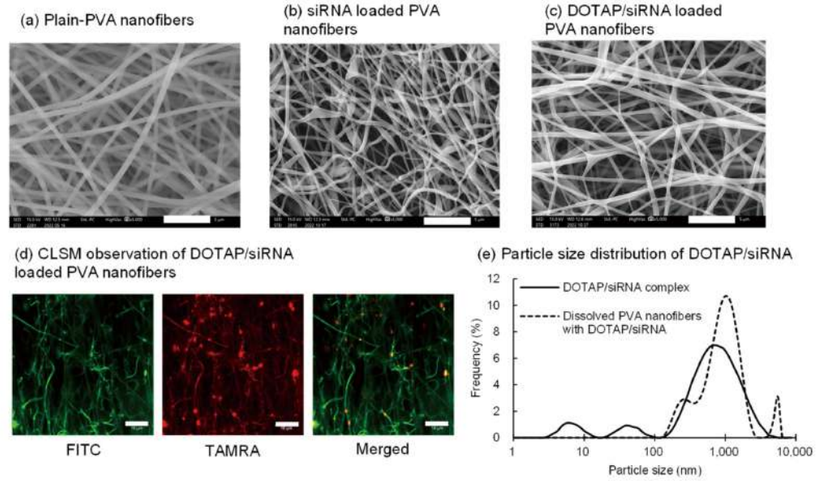

Figure 1. Characteristics of the DOTAP/siRNA-containing PVA nanofibers (N/P ratio = 5) prepared by electrospinning of 8% (*w*/*w*) PVA (EG-40P) aqueous solution. The nanofibers were prepared using Method A. (**a**) Scanning electron microscope (SEM) images of the PVA nanofibers without encapsulated siRNA (plain PVA nanofibers). (**b**) SEM images of the PVA nanofibers containing naked siRNA (N/P ratio = 0). (**c**) SEM images of the DOTAP/siRNA-containing PVA nanofibers. Scale bar: 5 µm. (**d**) Confocal laser scanning microscope images of the DOTAP/TAMRA-siRNA-containing FITC-PVA nanofibers. Scale bar: 10 µm. (**e**) Comparison of the particle size distribution of the DOTAP/siRNA complexes immediately after preparation and after being released from the PVA nanofibers, which were completely dissolved in water.

3.2. RNAi Efficacy of the DOTAP/siRNA-Containing PVA Nanofibers

The RNAi efficacy of DOTAP/Luc-siRNA-containing nanofibers was evaluated based on the suppression of luciferase expression in A549-Luc cells (Figure 2a). As expected, nanofibers containing naked siRNA (N/P ratio = 0) did not show any inhibition. DOTAP/siRNA complexes at an N/P ratio of 5 inhibited luciferase expression by ~60%.

The two preparation methods of the PVA nanofibers tested are represented in Figure 2a. Method A involved mixing the DOTAP/siRNA complex with the PVA solution before spinning, whereas Method B entailed adding the DOTAP suspension and siRNA solution separately into the PVA solution. The morphology of the DOTAP/siRNA-containing PVA nanofibers was not notably affected by the methods (Figure S1). The effect of RNAi on A549-Luc cells was verified using both methods, which revealed no marked variation in the inhibition of luciferase expression. This finding suggests that bypassing the lipoplex preparation process and directly mixing cationic lipids with nucleic acids in a PVA aqueous solution facilitated siRNA delivery into the cells. In Method B, DOTAP and siRNA in the PVA solution could potentially form complexes. This approach simplified the nanofiber

fabrication process, which is critical for scaling up production. This study evaluated the impact of siRNA after 24 h of adding the samples to A549-Luc cells. However, the RNAi activity can vary with incubation time. Therefore, future studies should assess the time course of RNAi efficacy for DOTAP/siRNA released from PVA nanofibers.

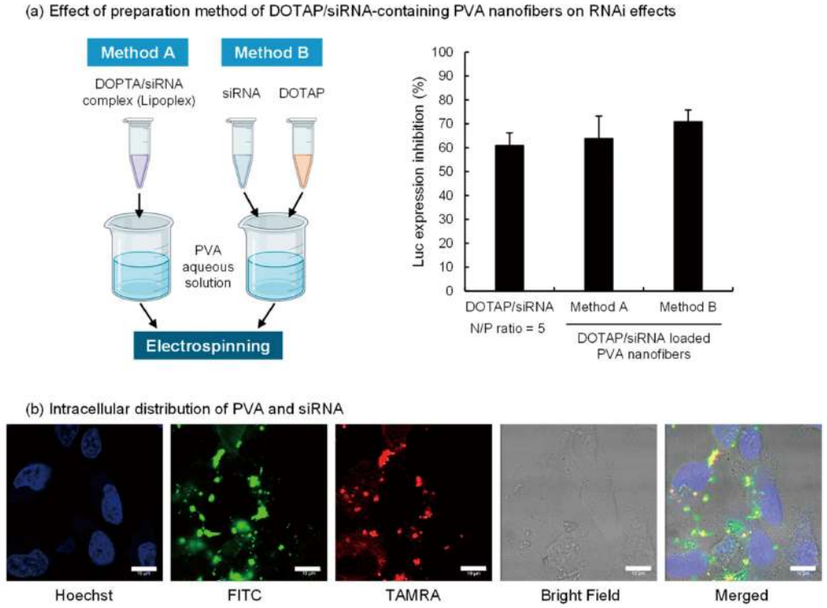

Figure 2. Inhibition of luciferase expression and intracellular distribution of DOTAP/siRNA complexes released from PVA (EG-40P) nanofibers. (**a**) The schematic diagram on the left illustrates the preparation methods of DOTAP/siRNA-containing PVA nanofibers (N/P ratio = 5), created with BioRender.com. The right side of the figure shows the effect of these different preparation methods on the gene expression inhibition rate in A549-Luc cells. The bars in the graph represent mean values ± SD (n = 3). (**b**) Intracellular distribution of FITC-PVA and TAMRA-siRNA in A549-Luc cells. CLSM images were taken after 4 h of incubation of cells with the completely dissolved DOTAP/siRNA-containing PVA nanofiber solution using FITC-PVA and TAMRA-siRNA in a serum-free medium. Scale bar: 10 μm.

Subsequently, the intracellular behavior of DOTAP/siRNA-containing PVA nanofibers prepared via Method A on A549-Luc cells was assessed using CLSM (Figure 2b). Method A, which involves preparing the DOTAP/siRNA complexes before adding them to the PVA solution, was adopted to evaluate the intracellular behavior of DOTAP/siRNA complexes released from PVA nanofibers. Considering the possibility that PVA remained attached to the released DOTAP/siRNA complexes, the intracellular distribution of FITC-PVA and TAMRA-siRNA was ascertained. A549-Luc cells were exposed to a serum-free medium containing PVA nanofibers, followed by a 4 h incubation and observation with CLSM. The results indicated siRNA within the cytoplasm of A549-Luc cells, with PVA detected inside the cells, often colocalized with siRNA. This observation suggests that the DOTAP/siRNA complexes with adhered PVA were internalized into the cells via endocytosis. Thus, the

grade of PVA, including its degrees of polymerization and hydrolysis, influences the interactions between DOTAP/siRNA complexes and cells [34].

3.3. Impact of PVA Grades on the DOTAP/siRNA-Containing PVA Nanofibers

The nanofibers containing DOTAP/siRNA (N/P ratio = 5) were fabricated by employing the electrospinning method A with PVAs of different degrees of polymerization, saponification, and charges to investigate the impact of various types of PVA on the intracellular delivery of nucleic acids. Table 1 lists the characteristics of the PVAs used in this experiment. As shown in Figure 2a, there was no remarkable difference in RNAi efficacy between Methods A and B. Therefore, Method A was adopted for this study. However, for future investigations, it will be necessary to prepare nanofibers with different PVA grades by employing Method B and evaluating their RNAi efficacy. Figure 3 presents the SEM images of the nanofibers fabricated with various PVAs. With the exception of EG-05P, an 8% solution of PVA was used for electrospinning. Previous studies by our group have shown that the relatively low molecular weight of EG-05P results in a lesser viscosity of 8%, making it unsuitable for spinning [22]. Therefore, a 20% solution was used for EG-05P in this study (Table 1). Nanofibers were spun from all types of PVA and collected as mats from the plate collector. The nanofibers from NH-18 (fully hydrolyzed) and K-434 (cationic PVA) exhibited bead-on-string structures (Figure 3d,e).

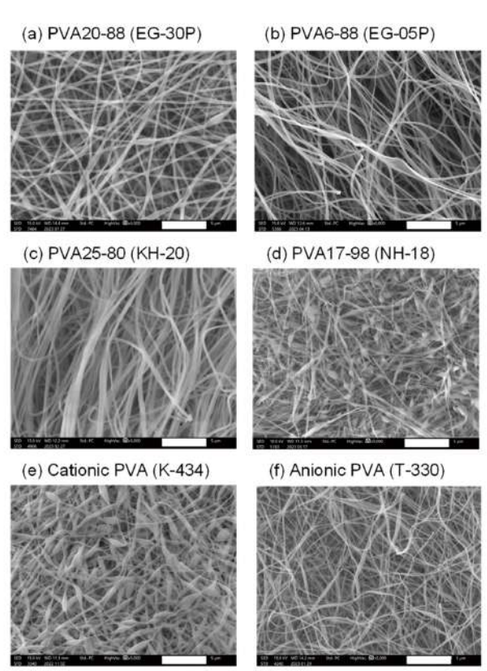

Figure 3. Scanning electron microscope (SEM) images of DOTAP/siRNA-containing PVA nanofibers (N/P ratio = 5) using different grades of PVA. PVA nanofibers were prepared using Method A. Scale bar: 5 μm. (**a**) PVA20-88 (EG-30P), (**b**) PVA6-88 (EG-05P), (**c**) PVA25-80 (KH-20), (**d**) PVA17-98 (NH-18), (**e**) Cationic PVA (K-434), (**f**) Anionic PVA (T-330). Each SEM image corresponds to the nanofibers listed in Table 1.

Figure 4 demonstrates the effects of the DOTAP/siRNA-containing PVA nanofibers on the suppression of luciferase expression in A549-Luc cells. PVA6-88 (EG-05P) demonstrated a lower inhibition rate. Compared to other formulations, with PVA6-88, the higher polymer concentration (20%) in the solution before electrospinning resulted in a greater amount of PVA in the nanofibers. When these PVA6-88 nanofibers were dissolved, more PVA would adhere to the DOTAP/siRNA complexes. This affinity enhanced the presence of PVA on the complexes, which may hinder their interaction with cells, reducing the RNAi activity.

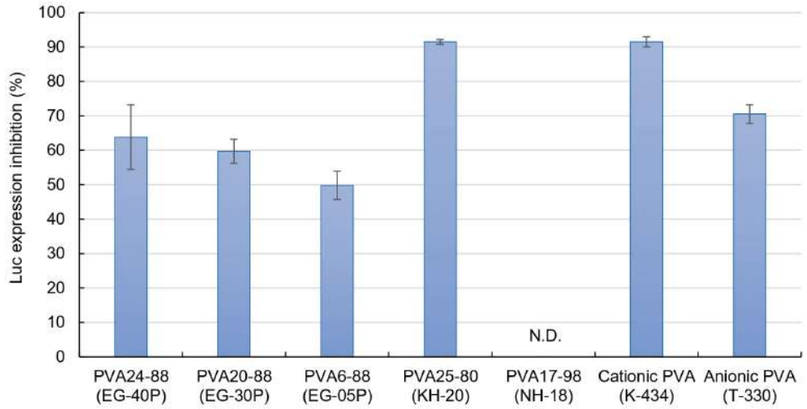

Figure 4. The efficacy of DOTAP/siRNA-containing PVA fibers (N/P ratio = 5) in inhibiting luciferase expression. Different grades of PVA nanofibers were prepared using Method A. Each bar represents the mean value ± SD (n = 4).

Conversely, the RNAi effect of PVA25-80 (KH-20), a PVA with a low degree of hydrolysis, was 91.5 ± 0.7%, which was higher compared with the partially hydrolyzed PVAs. Low-hydrolysis PVAs contain more acetyl groups, which enhances their hydrophobicity. The elevated presence of hydrophobic PVA on the surface of the DOTAP/siRNA complexes facilitated the cell internalization of the complexes. The fully hydrolyzed PVA17-98 (NH-18) did not inhibit luciferase expression. Fully hydrolyzed PVA has lower water solubility owing to the intermolecular and intramolecular hydrogen bonding among the hydroxyl groups of the PVA molecules being more substantial than their interactions with water, limiting the release of DOTAP/siRNA from the nanofibers [35].

Cationic (K-434) and anionic (T-330) PVAs are mainly used in industrial applications but not as pharmaceutical excipients [36–38]. The suppression of luciferase expression was relatively high for these types of PVA, particularly cationic PVAs. The presence of charged PVA near the surface of the DOTAP/siRNA complexes enhances their interaction with the cells. For instance, fabricating nanofibers blended with cationic polymers such as chitosan and PVA can potentially maximize the efficacy of intracellular siRNA delivery [10,29].

Assessment of the cytotoxicity of cationic and anionic PVA nanofibers revealed a slight decline in the cell viability of cationic PVA (Figure 5). Although cationic PVA allowed efficient intracellular siRNA delivery, the potential for marginal cell damage suggests further consideration.

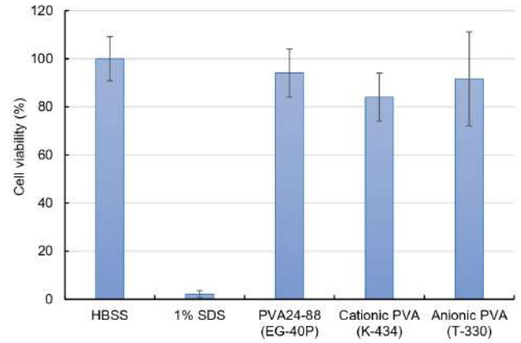

Figure 5. Cytotoxicity evaluation of A549-Luc cells treated with DOTAP/siRNA-containing PVA nanofibers (N/P ratio = 5) by employing the WST-8 assay. A549-Luc cells were exposed to various samples containing 100 nM siRNA for 4 h, and their viability was measured. As a positive control, 1% sodium dodecyl sulfate was also evaluated. Bars in the graph represent the mean values ± SD (n = 4–5).

4. Conclusions

This study aimed to develop a nucleic acid drug delivery system using electrospun PVA nanofibers by encapsulating DOTAP/siRNA complexes within the nanofibers and elucidating the characteristics of siRNA-encapsulated nanofibers, including their RNAi activity. It was demonstrated that electrospinning efficiently encapsulated DOTAP/siRNA complexes within the PVA nanofibers. Stable inhibition of luciferase expression was observed, even when the DOTAP/siRNA complex preparation process was bypassed by directly adding each component to the PVA solution. Furthermore, DOTAP/siRNA complexes released from the PVA nanofibers bore PVA residues on their surfaces, suggesting that the properties of PVA may influence interactions between the complexes and cells. Therefore, the degree of hydrolysis and polymerization, or the grade of PVA, affected luciferase expression inhibition, with a higher RNAi activity observed using low hydrolysis degree PVA. Although modified PVA carrying a charge is not currently authorized as a pharmaceutical excipient, the intense inhibition of luciferase expression demonstrated that blending charged polymers with PVA could potentially create more efficient nanofibers for the delivery of nucleic acid drugs to cells.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nano14131083/s1, Figure S1: Comparison of SEM images of DOTAP/siRNA-containing PVA nanofibers prepared using different methods (Method A and B) as shown in Figure 2. PVA (EG-40P) was used, and the N/P ratio of DOTAP/siRNA was 5. The SEM image on the left (Method A) corresponds to Figure 1c in the main text.

Author Contributions: Conceptualization, K.T.; Methodology, M.K., E.Y., T.I. and K.T.; Validation, M.K. and E.Y.; Formal analysis, M.K. and K.T.; Investigation, M.K. and K.H.; Resources, K.H. and K.T.; Data curation, M.K. and K.T.; Writing—original draft, M.K. and K.T.; Writing—review & editing, M.K., K.H., E.Y., T.I. and K.T.; Supervision, K.H. and K.T.; Project administration, K.T.; Funding acquisition, K.T. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are contained within the article and Supplementary Materials.

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Conflicts of Interest: K.H. reports a relationship with the Mitsubishi Chemical Corporation, which includes employment. K.H. and K.T. have patents #Japanese Patent Application No. 2023-51356 pending with Mitsubishi Chemical Corporation. Author K.H. was employed by the company Mitsubishi Chemical Corporation. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Controlled release of lysozyme using polyvinyl alcohol-based polymeric nanofibers generated by electrospinning

815

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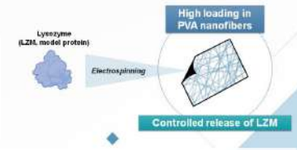
Introduction

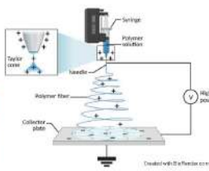
Polymeric nanofibers generated via electrospinning offer a promising platform for drug delivery systems. This study examines the application of electrospun polyvinyl alcohol (PVA) nanofibers for controlled lysozyme (LZM) delivery. By using various PVA grades, such as the degree of polymerization/hydrolysis, this study investigates their influence on nanofiber morphology and drug-release characteristics. Furthermore, we attempted to coat PVA/LZM nanofibers with hydrophobic polycaprolactone (PCL) using coaxial needle electrospinning to achieve extended LZM release.



Materials and methods

PVA grades with different degrees of polymerization and hydrolysis used to prepare LZM-containing nanofibers

Abbreviation	Trade name (GOHSENOL™ series)	Degree of polymerization	Degree of hydrolysis (mol%)
PVA24-88	EG-40P	2400	88
PVA6-88	EG-05P	600	88
PVA25-80	KH-20	2500	80



Electrospinning conditions

LZM-containing PVA monolithic nanofibers

The LZM-containing PVA solution in water was loaded into a syringe via a 22G needle. Randomly oriented nanofibers were electrospun by applying 10 kV with a high-voltage supplier. The standing plate collector was located 12 cm from the needle, and by using a syringe pump, the solution was loaded at a feed rate of 0.5 mL/h.

LZM-containing PVA/PCL core–shell nanofibers

The shell solution was prepared by dissolving 1.2 g of PCL in 10 g of chloroform/DMF solution. The core solution was prepared by dissolving 0.2 g of PVA EG-40P in 9.8 g of distilled water, followed by complete dissolution of 0.1 g of LZM. A coaxial spinneret with a 26-gauge core needle and a 20-gauge shell needle was connected to the syringe. By using two syringe pumps, the core solution was pumped at a flow rate of 0.2 mL/h and the shell solution at a flow rate of 6.5 mL/h.

Effect of the PVA grades on LZM release from PVA nanofibers

LZM-containing PVA monolithic nanofibers were prepared by electrospinning using various grades of PVA. The high drug encapsulation efficiency was approximately100. The absence of LZM inactivation in PVA nanofibers following the electrospinning process was confirmed.

PVA24-88 PVA6-88 PVA25-80

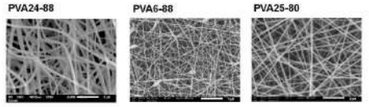


Fig. 1. Scanning electron microscope (SEM) images of LZM-containing PVA nanofibers with different grades of PVA (Weight ratio: LZM/PVA = 1/1). Scale bar, 5 μm.

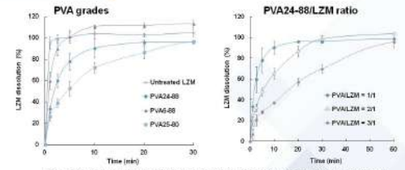


Fig. 2. LZM dissolution profiles of LZM-containing PVA monolithic nanofibers with different PVA grades (left) and PVA24-88/LZM ratio (right). Values are expressed as the mean ± SD (n = 3).

PVA grades affected initial dissolution of lysozyme (PVA solubility dependent)

PCL/PVA core–shell nanofiber to release LZM over the long term

In the previous section, we demonstrated that the release of LZM from PVA nanofibers could be controlled by different grades of PVA and LZM content. However, controlling long-term release with PVA alone would be challenging because LZM release from nanofibers depends on the solubility of PVA itself. We attempted to coat PVA/LZM nanofibers with hydrophobic PCL using coaxial needle electrospinning to achieve extended LZM release.

Core–shell nanofibers demonstrated sustained release of LZM for >3 d. Nanofibers with a shell layer of hydrophobic PCL remained in the vessel following the dissolution test. The LZM dissolution profile followed the Higuchi model, suggesting that release is controlled via drug diffusion in the PCL matrix of the shell layer. During the 3-d period, the mechanism for erosion of the PCL shell layer may have a limited effect on the release of LZM.

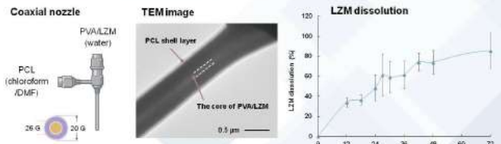


Fig. 3. LZM-containing PCL/PVA core–shell nanofibers prepared by coaxial electrospinning (left). TEM image of PCL/PVA core–shell nanofibers (middle). The white dotted line in the TEM image is the boundary of the core–shell layer. Dissolution test of LZM from nanofibers (right). Values are expressed as the mean ± SD (n = 3).

Long-term sustained release of lysozyme (Drug diffusion dependence in the PCL layer)

Conclusions

One-step preparation of PVA nanofibers with high content of LZM in the 200–400 nm range by electrospinning was achieved easily. The degree of polymerization of PVA impacted morphology of the nanofiber, whereas the degree of hydrolysis of PVA and LZM content affected the release of LZM from the nanofibers. PVA solubility-dependent control of LZM release from the nanofibers was achieved. The preparation of PVA nanofibers coated with PCL (core–shell nanofibers) via coaxial electrospinning permitted the long-term control of LZM release via the LZM diffusion mechanism in the PCL layer matrix. Nanofibers that contain biopharmaceuticals such as LZM can be generated by electrospinning with PVA as a base material for nanofibers, and drug release could be achieved by modifying the pharmaceutical formulation to achieve the desired drug release.

Reference: Ogawa, R et al., *Chem Pharm Bull*, 72, 324-329 (2024) . https://doi.org/10.1248/cpb.c24-00024.

高分子ナノファイバーを活用した創剤・DDS開発

Pharmaceutical formulation and DDS development using polymeric nanofibers

製剤技術
A-08

岐阜薬科大学　ナノファイバー創剤学寄附講座
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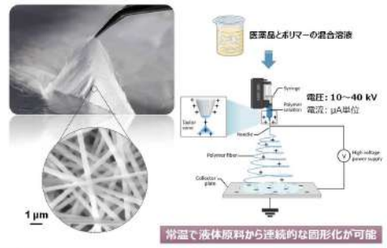
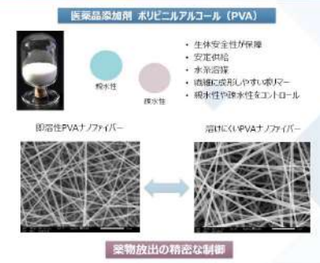
研究背景

エレクトロスピニング法により調製する高分子ナノファイバーは、基剤を適切に選択することにより、低分子、ペプチド、タンパク、核酸など様々なモダリティを高効率に担持することができる。ナノファイバーのシートをそのまま貼付剤として用いたり、加工して内服薬としても利用できるなど幅広いアプリケーションが期待できる。

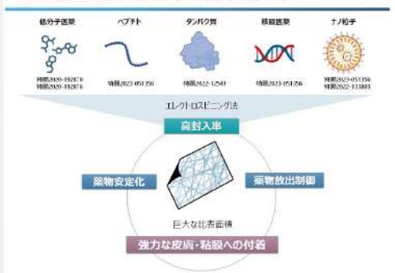
エレクトロスピニング法によるナノファイバー設計



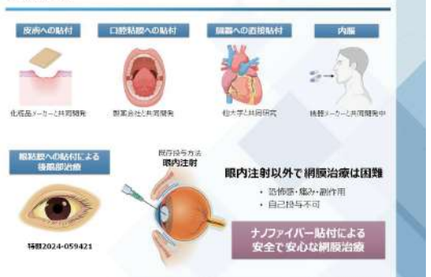
安全でシンプルな素材による精密制御



様々なモダリティの製剤化プラットフォーム



応用事例



ナノファイバー眼球貼付による網膜への薬物デリバリー

網膜疾患
- 加齢黄斑変性症（AMD）や糖尿病網膜症などは高齢者に多く失明の原因となる重篤疾患

本件に関する連絡先

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- 医薬品に限らず、化粧品や機能性食品成分にもナノファイバーを適用する研究をしています
- ナノファイバー技術の医薬品、機能性食品、香粧品分野への応用に興味がある方は、産官学問わずお気軽にお問い合わせください

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A61K 9/51	*(2006.01)*	A61K	9/51	
A61K 47/32	*(2006.01)*	A61K	47/32	
A61K 47/24	*(2006.01)*	A61K	47/24	
A61K 9/70	*(2006.01)*	A61K	9/70　401	

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1．TWEEN

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