SYNTHESIS AND APPLICATIONS OF NON-VIRAL GENE VECTORS

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by

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Dedicated to my father

Losing my father during thesis writing will always sting, yet everything I do now is a tribute to his memory, celebrating his life.

Supervisor Certification

This is to certify that the thesis entitled "**Synthesis and applications of non-viral gene vectors**" being submitted by **Mr. Manoj Kumar Sharma** to the Indian Institute of Technology Delhi and The University of Queensland for the award of degree of **Doctor of Philosophy** is a record of *bonafide* research work carried out by him. **Mr. Manoj Kumar Sharma** has worked under our guidance and supervision and has fulfilled the requirements for the submission of this thesis, which to our knowledge has reached the requisite standard. The results contained in this thesis are original and have not been submitted, in part or full, to any other University or Institute for the award of any other degree or diploma.

26/07/2024

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Abstract

A nanocarrier designed for cellular delivery plays a crucial role in encapsulating, safeguarding, and efficiently transporting payloads across cell membranes, facilitating their entry into the cytosol. Nanoparticle-based drug delivery systems (DDS) have emerged as a promising avenue for drug therapy, leveraging nanoparticles to enhance bioavailability, controlled release, and prolong the circulation time of drugs, thereby increasing efficiency and safety. Mesoporous silica nanoparticles (MSN)-based carriers have garnered substantial attention due to their high porosity, biocompatibility, and facile particle size control. In addition to porosity, incorporating spiky nanotopography on MSNs introduces unique properties for bacteria adhesion, intracellular delivery, and plasmid DNA (pDNA) protection. Despite their advantages, MSN-based DDS face challenges such as low loading capacity and premature release of anticancer drugs. Surface modification of MSN offers a promising strategy for further enhancing drug loading, controlled release, and therapeutic efficacy. An innovative strategy involves creating virus-mimicking nanoparticles designed to safeguard therapeutic cargo from enzymatic degradation and premature release, all while accommodating a substantial loading capacity for biomolecules. The concept of crafting spiky silica nanoparticles with solid spikes on their surface, resembling viruses, is particularly fascinating. Although silica inherently lacks stimuli-responsive properties, incorporating it with metal-organic frameworks (MOFs) like ZIF-8/ZIF-90 can impart acid-responsive characteristics to the nanocarrier.

The primary aim of this thesis is to design non-virus-based nanocarriers that mimic nature, with significant relevance in diverse biological applications such as drug delivery, protein delivery, gene delivery, and antimicrobial activities. The nanocarrier system utilizing MSN for efficient cellular delivery of therapeutic payloads, addressing challenges of low loading capacity, high cytotoxicity and premature release through surface modification techniques.

Our results indicate a remarkable loading capacity of SNP-ZIF-90 for doxorubicin, surpassing that of bare SNPs, attributed to the formation of Schiff base interactions. Furthermore, SNP-ZIF-90 demonstrates acid-responsive behavior, with higher percentages of drug release at acidic pH levels. Importantly, the developed system also facilitates the endosomal escape of chemotherapeutic drug molecules and shows a substantial 6.5 and 3 times reduction in the half-maximal inhibitory concentration (IC50) compared to free doxorubicin (Dox) and SNP-Dox, respectively, indicating enhanced therapeutic efficacy. Similarly, we utilized SNP-ZIF-8 for the delivery of mRNA, embedding SNP mesopores with 27% ZIF-8 nanocrystals while

retaining the spiky morphology. This design ensures proficient mRNA loading, provides protection against enzymatic degradation, and induces endosomal escape. Our findings demonstrate that SNP-ZIF-8 exhibits minimal cytotoxicity in various cell lines and superior cellular uptake compared to ZIF-8. Additionally, in-vitro mRNA transfection rates are 1.5 times higher than those achieved with SNPs alone and show a 1.2-fold improvement over the commercially available Lipofectamine RNAmax. Expanding the scope of synthesized nanoparticles in treating gram-negative bacterial infections, we demonstrated that SNP-ZIF-8 loaded with the protein Lysozyme can adhere to the bacterial cell membrane through multivalent interactions. Zinc ions released from ZIF-8 near the cell membrane increase bacterial cell membrane permeability and reactive oxygen species (ROS) generation. The overproduction of extracellular ROS assists in reaching Lysozyme to the inner bacterial cell wall, lysing the peptidoglycan layer, resulting in the release of cytosol contents, and effectively killing H. pylori. In summary, this thesis presents the design and evaluation of non-virus-based nanocarriers, particularly SNP-ZIF-90 and SNP-ZIF-8, for efficient drug and mRNA delivery, as well as antimicrobial activities. These nanocarriers demonstrate remarkable loading capacities, acid-responsive behavior, and enhanced therapeutic efficacy, addressing challenges such as low loading capacity and premature release. Future directions include further exploration of surface modification techniques to enhance drug delivery efficiency and expanding applications to other biological processes.

सार (Abstract in Hindi)

सेलुलर डिलीवरी के लिए डिज़ाइन किया गया एक नैनोकैरियर कोशिका झिल्ली में पेलोड को एनकैप्सुलेट करने, सुरक्षित रखने और कुशलतापूर्वक परिवहन करने में महत्वपूर्ण भूमिका निभाता है, जिससे साइटोसोल में उनके प्रवेश की सुविधा मिलती है। नैनोकण-आधारित दवा वितरण प्रणाली (डीडीएस) दवा चिकित्सा के लिए एक आशाजनक अवसर के रूप में उभरी है, जो जैवउपलब्धता बढ़ाने, नियंत्रित रिलीज और दवाओं के परिसंचरण समय को बढ़ाने के लिए नैनोकणों का लाभ उठाती है, जिससे दक्षता और सुरक्षा बढ़ती है। मेसोपोरस सिलिका नैनोकणों (एमएसएन)-आधारित वाहकों ने अपनी उच्च सरंध्रता, जैव अनुकूलता और सुस्पष्ट कण आकार नियंत्रण के कारण पर्याप्त ध्यान आकर्षित किया है। सरंध्रता के अलावा, एमएसएन पर स्पाइकी नैनोटोपोग्राफी को शामिल करने से बैक्टीरिया आसंजन, इंट्रासेल्युलर डिलीवरी और प्लास्मिड डीएनए (पीडीएनए) सुरक्षा के लिए अद्वितीय गुण पेश होते हैं। अपने फायदों के बावजूद, एमएसएन-आधारित डीडीएस को कम लोडिंग क्षमता और कैंसर रोधी दवाओं के समय से पहले जारी होने जैसी चुनौतियों का सामना करना पड़ता है। एमएसएन का सतही संशोधन दवा लोडिंग, नियंत्रित रिलीज और चिकित्सीय प्रभावकारिता को और बढ़ाने के लिए एक आशाजनक रणनीति प्रदान करता है। एक नवोन्मेषी रणनीति में वायरस-नकल करने वाले नैनोकणों का निर्माण शामिल है, जो चिकित्सीय कार्गो को एंजाइमी क्षरण और समय से पहले रिलीज से बचाने के लिए डिज़ाइन किया गया है, साथ ही बायोमोलेक्युलस के लिए पर्याप्त लोडिंग क्षमता को भी समायोजित करता है। उनकी सतह पर वायरस जैसे दिखने वाले ठोस स्पाइक्स वाले स्पाइकी सिलिका नैनोकणों को तैयार करने की अवधारणा विशेष रूप से आकर्षक है। हालाँकि सिलिका में स्वाभाविक रूप से उत्तेजना-प्रतिक्रियाशील गुणों का अभाव है, इसे ZIF-8/ZIF-90 जैसे धातु-कार्बनिक ढांचे (MOF) के साथ शामिल करने से नैनोकैरियर को एसिड-प्रतिक्रियाशील विशेषताएँ प्रदान की जा सकती हैं।

इस थीसिस का प्राथमिक उद्देश्य गैर-वायरस-आधारित नैनोकैरियर्स को डिजाइन करना है जो प्रकृति की नकल करते हैं, दवा वितरण, प्रोटीन वितरण, जीन वितरण और रोगाणुरोधी गतिविधियों जैसे विविध जैविक अनुप्रयोगों में महत्वपूर्ण प्रासंगिकता के साथ। नैनोकैरियर प्रणाली चिकित्सीय पेलोड की कुशल सेलुलर डिलीवरी के लिए एमएसएन का उपयोग करती है, सतह संशोधन तकनीकों के माध्यम से कम लोडिंग क्षमता, उच्च साइटोटॉक्सिसिटी और समय से पहले रिलीज की चुनौतियों का समाधान करती है।

हमारे परिणाम डॉक्सोरूबिसिन के लिए एसएनपी-जेडआईएफ-90 की एक उल्लेखनीय लोडिंग क्षमता का संकेत देते हैं, जो कि नंगे एसएनपी से अधिक है, जो शिफ बेस इंटरैक्शन के गठन के लिए जिम्मेदार

है। इसके अलावा, एसएनपी-जेडआईएफ-90 अम्ल-उत्तरदायी व्यवहार प्रदर्शित करता है, जिसमें अम्लीय पीएच स्तर पर दवा रिलीज का प्रतिशत अधिक होता है। महत्वपूर्ण रूप से, विकसित प्रणाली कीमोथेराप्यूटिक दवा अणुओं के एंडोसोमल पलायन की सुविधा भी देती है और मुक्त डॉक्सोरूबिसिन (डॉक्स) और एसएनपी-डॉक्स की तुलना में क्रमशः आधे-अधिकतम निरोधात्मक एकाग्रता (IC50) में 6.5 गुना और 3 गुना कमी दिखाती है। बढ़ी हुई चिकित्सीय प्रभावकारिता का संकेत। इसी तरह, हमने एमआरएनए की डिलीवरी के लिए एसएनपी-जेडआईएफ-8 का उपयोग किया, स्पाइकी आकारिकी को बनाए रखते हुए एसएनपी मेसोपोर को 27% जेआईएफ-8 नैनोक्रिस्टल के साथ एम्बेड किया। यह डिज़ाइन कुशल एमआरएनए लोडिंग सुनिश्चित करता है, एंजाइमैटिक क्षरण के खिलाफ सुरक्षा प्रदान करता है, और एंडोसोमल एस्केप को प्रेरित करता है। हमारे निष्कर्ष दर्शाते हैं कि SNP-ZIF-8 विभिन्न सेल लाइनों में न्यूनतम साइटोटॉक्सिसिटी और ZIF-8 की तुलना में बेहतर सेलुलर अवशोषण प्रदर्शित करता है। इसके अतिरिक्त, इन-विट्रो एमआरएनए ट्रांसफ़ेक्शन दर अकेले एसएनपी के साथ प्राप्त की तुलना में 1.5 गुना अधिक है और व्यावसायिक रूप से उपलब्ध लिपोफ़ेक्टामाइन आरएनएमैक्स की तुलना में 1.2 गुना सुधार दिखाता है। ग्राम-नेगेटिव जीवाणु संक्रमण के उपचार में संश्लेषित नैनोकणों के दायरे का विस्तार करते हुए, हमने प्रदर्शित किया कि प्रोटीन लाइसोजाइम से भरपूर एसएनपी-जेडआईएफ-8 बहुसंयोजी अंतःक्रियाओं के माध्यम से जीवाणु कोशिका झिल्ली से चिपक सकता है। कोशिका झिल्ली के पास ZIF-8 से निकलने वाले जिंक आयन बैक्टीरिया कोशिका झिल्ली पारगम्यता और प्रतिक्रियाशील ऑक्सीजन प्रजातियों (आरओएस) पीढ़ी को बढ़ाते हैं। बाह्यकोशिकीय आरओएस का अधिक उत्पादन लाइसोजाइम को आंतरिक जीवाणु कोशिका दीवार तक पहुंचाने में सहायता करता है, पेप्टिडोग्लाइकन परत को नष्ट करता है, जिसके परिणामस्वरूप साइटोसोल सामग्री निकलती है, और एच. पाइलोरी को प्रभावी ढंग से मार देती है। संक्षेप में, यह थीसिस कुशल दवा और एमआरएनए वितरण के साथ-साथ रोगाणुरोधी गतिविधियों के लिए गैर-वायरस-आधारित नैनोकैरियर्स, विशेष रूप से एसएनपी-जेडआईएफ-90 और एसएनपी-जेडआईएफ-8 के डिजाइन और मूल्यांकन को प्रस्तुत करती है। ये नैनोकैरियर कम लोडिंग क्षमता और समय से पहले रिलीज जैसी चुनौतियों का समाधान करते हुए उल्लेखनीय लोडिंग क्षमता, एसिड-उत्तरदायी व्यवहार और बढ़ी हुई चिकित्सीय प्रभावकारिता प्रदर्शित करते हैं। भविष्य की दिशाओं में दवा वितरण दक्षता बढ़ाने और अन्य जैविक प्रक्रियाओं में अनुप्रयोगों का विस्तार करने के लिए सतह संशोधन तकनीकों की और खोज शामिल है।

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

I acknowledge that an electronic copy of my thesis must be lodged with the University Library and, subject to the policy and procedures of The University of Queensland, the thesis be made available for research and study in accordance with the Copyright Act 1968 unless a period of embargo has been approved by the Dean of the Graduate School.

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Publications included in this thesis

Sharma, et al. "ZIF-90-decorated silica nanoparticles with a spiky surface: a novel approach to drug delivery." *New Journal of Chemistry* 48.13 (2024): 5760-5768.

Submitted manuscripts included in this thesis

None

Other publications during candidature

- Yadav, Priyanka, Anirban Das, **Manoj Kumar Sharma**, Soumen Ash, and Ashok Kumar Ganguli. A biodegradable polymer-assisted efficient and universal exfoliation route to a stable few layer dispersion of transition metal dichalcogenides. Materials Chemistry and Physics (2021): 125347.
- Kumari, Beeta, Manisha Yadav, **Manoj Kumar**, Pratibha Kushwaha, N. Prakash Prabhu, and Rajesh Kumar. Determinants for macromolecular crowding-induced thermodynamic stabilization of acid-denatured cytochrome c to molten globules. Journal of Molecular Liquids 387 (2023): 122608.

Contributions by others to the thesis

Contributor	Statement of contribution
Chengzhong Yu	Design of the project, analysis and interpretation of data, drafting and revision
Ashok Kumar Ganguli	Design of the project, analysis and revision
Jie Tang	conceptualization, methodology, experiment design, review & editing
Hao Song	methodology, experiment design.

All chapters

Editorial suggestions were provided by the candidate's supervisors, Prof. Chengzhong Yu and Prof. Ashok Kumar Ganguli and PhD committee, Prof. Sunil Khare, Prof. Sampa Saha, and Dr. Jie Tang.

Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research involving human or animal subjects

No animal or human subjects were involved in this research.

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Keywords

Nanoparticles, Drug Delivery, Spiky, Gene delivery, pH responsive, SNP-ZIF-8 and SNP-ZIF-90

Australian and New Zealand standard research classifications (ANZSRC)

[Provide data that links your thesis to the disciplines and discipline clusters in the Federal Government's Excellence in Research for Australia (ERA) initiative.
Please allocate the thesis a **maximum of 3** Australian and New Zealand Standard Research Classifications (ANZSRC) codes at the **6 digit level** and include the descriptor and a percent weighting for each code. Total percent must add to 100.
Example:
ANZSRC code: 100708, Nanomaterials, 50%
ANZSRC code: 100709, Nanomedicine, 30%
ANZSRC code: 090301, Biomaterials, 20%

Fields of research classification

FoR code: 4018, Nanotechnology, 60%
FoR code: 0699, Other Biological Sciences, 4z0%]

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