DOI: 10.25205/978-5-4437-1843-9-229

DEVELOPMENT OF ZIF-8 NANOCARRIERS FOR TARGETED INTRACELLULAR DELIVERY OF NUCLEIC ACIDS

Z. Hussein[1,2], S. R. Da Silva[2], I. P. Santos[2], J. P. Juste[2], G. B. González[2]

[1] *SCAMT Institute, ITMO University, Saint Petersburg*
[2] *CINBIO, University of Vigo, Spain*

✉ zain98hussein@gmail.com

Abstract

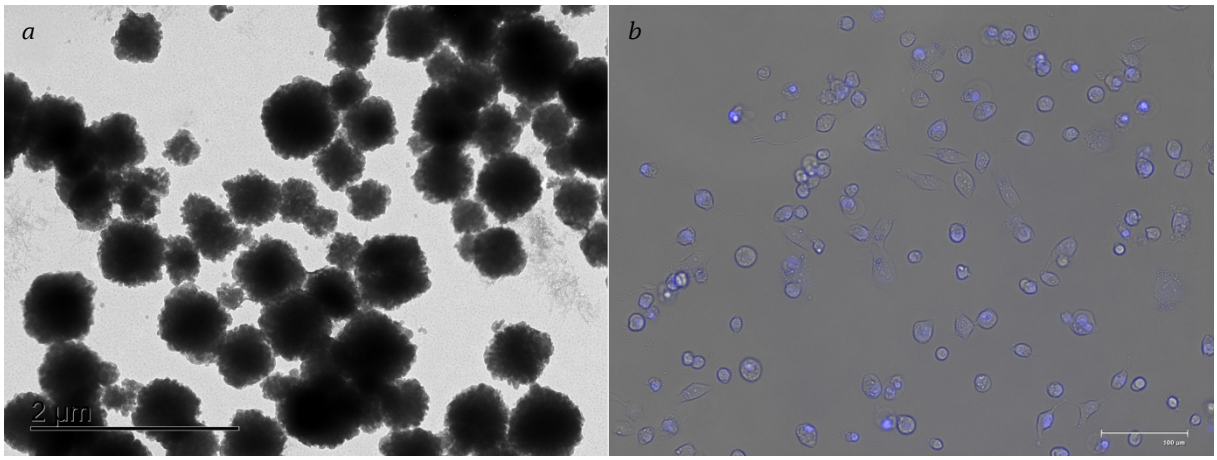
Gene therapy represents a promising strategy for cancer treatment, where the effective gene delivery plays a main role in the outcomes. Recently, metal–organic frameworks (MOFs) have gained attention as carriers for intracellular delivery of nucleic acids. In this work, we focused on the delivery of a GFP-expressing plasmid using MOFs and its polyethyleneimine (PEI)-coated form, evaluating their potential as safe, accessible platforms for nucleic acid delivery.

Gene therapy offers a promising avenue for cancer treatment, but its success largely depends on the efficiency and safety of gene delivery systems. While viral vectors provide high transfection efficiency, they pose risks such as immune responses and organ toxicity. Nonviral vectors are safer and more biocompatible but often suffer from low specificity and transfection efficiency. Thus, the development of effective and safe nonviral delivery systems remains a critical challenge in advancing gene therapy [1].

Zeolitic imidazolate framework-8 (ZIF-8), a member of the MOFs family, is being actively studied as a potential carrier for nucleic acid delivery. Its high surface area, adjustable pore structure, and ease of functionalization make it well-suited for encapsulating and releasing nucleic acids. The capacity for structural modification and pH-responsive release further enhances its appeal for gene therapy and other nucleic acid-based therapeutic strategies [2, 3].

In this study, nanoparticles were synthesized by mixing 2-methylimidazole with zinc nitrate and incubating for 3 hours. Transmission electron microscopy (TEM) revealed particles of varying sizes, with an average length of 350 nm (see figure, *a*). Plasmid (pcDNA3.1-GFP) encapsulation was assessed via agarose gel electrophoresis, showing near-complete encapsulation, as indicated by the absence of plasmid bands in the supernatant and retention in the MOF wells.

To evaluate transfection efficiency, MDA-MB-231 cells were treated with ZIF-8-based carriers encapsulating either pcDNA3.1-GFP (pcDNA3.1-GFP@ZIF-8 and pcDNA3.1-GFP@ZIF-8-PEI) or Hoechst 33258 as a control for cellular uptake (Hoechst-33258@ZIF-8). After 24 hours, nuclear staining confirmed internalization of Hoechst-loaded ZIF-8 (see figure, *b*), while no staining was observed during the initial 4 hours, suggesting uptake occurred only after internal-



Transmission electron microscopy image showing ZIF-8 nanoparticles with heterogeneous size distribution (*a*), fluorescence microscopy image of MDA-MB-231 cells displaying successful nuclear staining after 24-hour incubation with Hoechst-33258@ZIF-8 (*b*)

ization, and no ZIF-8 were dissolved in the culture medium. Cells treated with pcDNA3.1-GFP@ZIF-8 showed no GFP expression, likely due to poor uptake caused by the material's near-neutral surface charge, as confirmed by zeta potential analysis. In contrast, cells treated with positively charged pcDNA3.1-GFP@ZIF-8-PEI showed low levels of GFP expression after 48 hours. Further optimization of used concentrations and transfection conditions is needed before comparing against commercial transfection agents.

The long-term objective is to adapt this system for therapeutic nucleic acids such as antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) for cancer gene therapy.

References

1. Salari R. et al. P53 Gene Therapy with ZIF-8 Metal-Organic Framework: A Platform in Cancer Gene Therapy // ACS Omega. 2025. Vol. 10, No. 11. P. 10891–10902.

2. Polash S.A. et al. Phase Characterization and Bioactivity Evaluation of Nucleic Acid-Encapsulated Biomimetically Mineralized ZIF-8 // ACS Appl Mater Interfaces. 2025. Vol. 17, No. 2. P. 3002–3012.

3. Sameni M. et al. ZIF-8 Nanoparticle: A Valuable Tool for Improving Gene Delivery in Sperm-Mediated Gene Transfer // Biol Proced Online. 2024. Vol. 26, No. 1. P. 4.