Acta Pharmaceutica Sinica B 2025;15(3):1709−1711

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

HIGHLIGHT

Liquid−liquid phase separation-assembled coacervate vesicles for biopharmaceutical delivery

KEY WORDS

Liquid−liquid phase separation;
Coacervate vesicles;
Delivery vehicle;
Oncolytic viruses

Liquid−liquid phase separation (LLPS) induces the formation of membrane-less droplet-like compartments through the segregation of biomolecules into distinct liquid phases within a solution, without the requirement for a surrounding lipid bilayer[1]. These membrane-less droplet-like compartments, which arise from LLPS, possess unique properties and functions essential for cellular organization, biomolecular condensation, and cellular signaling[2]. Recent advancements have increasingly enabled the utilization of these membrane—less compartments as delivery platforms[3]. However, LLPS assemblies often fail to meet long-term storage and application requirements due to challenges such as coalescence and disassembly[4].

Recently, a pioneering study published in *Nature Chemistry* by Gu's group[5] introduced an innovative coacervate vesicle delivery system leveraging liquid−liquid phase separation to enhance the efficient and targeted delivery of biopharmaceuticals (Fig. 1). Under an appropriate ratio of components, cholesterol-modified single-strand DNA (Chol-ssDNA) and histone molecules could self-assemble to generate highly stable liquid−liquid phase separation (LLPS)-assembled coacervate vesicles (CVs) through a simple mixing process. Unlike phospholipid-based membrane-bound vesicles, CVs lacked a surface membrane but featured a dense liquid layer surrounding a water-filled core. This unique configuration overcomes the limitations commonly associated with phospholipid membranes, such as low permeability and physical barriers, thereby enabling the effective encapsulation of viral particles, mRNA, cytokines, peptides, and various therapeutic agents while maintaining their biological activity.

Researchers employed oncolytic virus, specifically oncolytic adenovirus serotype 11 (ad11), as model drugs to explore the potential of CVs for drug delivery. *In vitro* experiments demonstrated that CVs effectively improved oncolytic virus infection in tumor cells, regardless of whether these cells expressed the requisite receptor for viral infections. The results highlighted that CVs notably enhanced the uptake of oncolytic virus in various tumor cell lines by up to 48-fold. Mechanistically, by evaluating the uptake of oncolytic virus by tumor cells in the presence of multiple inhibitors, Gu's group[5] identified distinct uptake pathways facilitated by CVs and elucidated the roles of macropinocytosis or phagocytosis in CV-cell interactions.

In a mouse model, the enhanced tumor infection facilitated by oncolytic viruses delivered *via* CVs not only extended tumor residence but also intensified their anti-tumor efficacy. Notably, the augmented anti-tumor effect induced by CVs was noticeable even when tumor volumes reached 500 mm^3. Additionally, CVs demonstrated favorable biosafety profiles. Throughout the treatment period, no significant organ damage or alterations in body weight were noted. Transient fluctuations in transaminase levels during treatment were observed in blood biochemical indexes, gradually normalizing over time. The pivotal aspect of oncolytic virus therapy lies in the induction of the anti-tumor immune response. CVs significantly facilitated the infiltration of $CD8^+$ T cells into tumors during oncolytic virus therapy. Furthermore, compared to direct intratumoral injection of oncolytic virus, the delivery of oncolytic virus *via* CVs effectively increased the

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2025.01.019

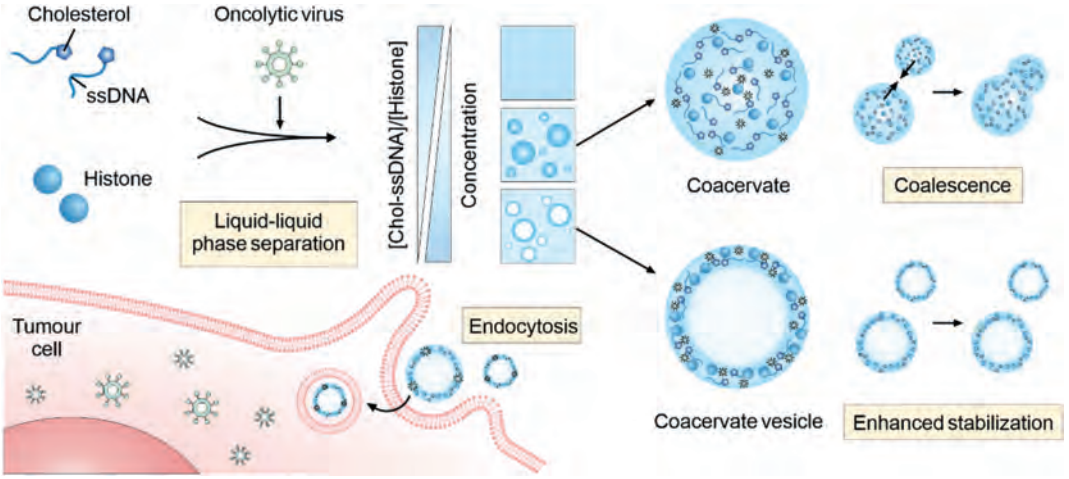

Figure 1 A schematic illustration of coacervate vesicle for oncolytic virus packing and intracellular delivery (copyright © 2025 Springer Nature).

presence of M1 macrophages within and decreased the abundance of M2 macrophages in tumor tissues, thus successfully modulating the tumor microenvironment.

LLPS carriers encounter significant limitations in drug delivery, including the potential instability of phase-separated structures and challenges in regulating drug release kinetics[4]. The shape and topological characteristics of these phase-separated carriers play a crucial role in influencing drug delivery efficiency and specificity, underscoring the necessity for a deeper comprehension of how these aspects impact drug transport within such systems[6]. Gu's group[5] have effectively optimized the morphology of condensate droplets by adjusting the ratio of Chol-ssDNA to histones, thereby enhancing the efficacy of oncolytic virus against tumors with low infectious receptor expression and inducing significant changes in the tumor microenvironment. Extensive research has delved into exploring the topology, physicochemical attributes, and intracellular delivery pathways associated with LLPS to delve further into the underlying mechanisms. This study has revealed that hydrophobicity, charge-based interactions, and electrostatic effects of multivalent interactions are pivotal determinants of the phase state and act as essential driving forces for condensation. This research, centered on the development of CVs based on LLPS and assembly mechanisms, introduces an innovative delivery platform for the efficient transportation of biotechnological drugs and cancer therapies. With its uncomplicated preparation process and outstanding biological safety profile, this carrier showcases significant promise as a versatile drug delivery system.

In contrast to the intricate extraction procedures associated with natural condensates and the instability often observed in membraneless compartments, the simplicity of operation and robust stability of CVs significantly enhance their potential for clinical translation. Moving forward, comprehensive preclinical studies are imperative to thoroughly evaluate the *in vivo* stability and safety of the vector before clinical implementation. Given the remarkable efficiency of CVs in delivering a wide range of biological drugs, its application is expected to extend beyond cancer treatment to include the delivery of proteins, mRNAs, and other therapeutic agents across various disease indications. The advancement of this technology holds the promise of providing substantial benefits to patients grappling with tumors or some refractory diseases.

Acknowledgments

This work was supported by the National Nature Science Foundation of China (No. 82073286), and State Key Laboratory of Neurology and Oncology Drug Development (No. SKLSIM-F-202418, China).

Author contributions

Zao Ji wrote the manuscript. Jianbin Bi, Hong-Xu Liu and Heran Li revised and supervised the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

References

1. Gao Y, Li X, Li P, Lin Y. A brief guideline for studies of phase-separated biomolecular condensates. *Nat Chem Biol* 2022;**18**:1307−18.
2. Astoricchio E, Alfano C, Rajendran L, Temussi P, Pastore A. The wide world of coacervates: from the sea to neurodegeneration. *Trends Biochem Sci* 2020;**45**:706−17.
3. Sun Y, Lau S, Lim Z, Chang S, Ghadessy F, Partridge A, et al. Phase-separating peptides for direct cytosolic delivery and redox-activated release of macromolecular therapeutics. *Nat Chem* 2022;**14**:274−83.
4. Abbas M, Law J, Grellscheid S, Huck W, Spruijt E. Peptide-based coacervate-core vesicles with semipermeable membranes. *Adv Mater* 2022;**34**:e2202913.
5. Wen P, Huang HW, Zhang RZ, Zheng HQ, Liang TXZ, Zhuang C, et al. Coacervate vesicles assembled by liquid−liquid phase separation improve delivery of biopharmaceuticals. *Nat Chem* 2025;**17**:279−88.

6. Mura S, Nicolas J, Couvreur P. Stimuli-responsive nanocarriers for drug delivery. *Nat Mater* 2013;**12**:991−1003.

Zao Ji[a,b,c], Jianbin Bi[d,*], Hong-Xu Liu[e,*], Heran Li[f,*]
[a]*Department of Surgical Oncology and General Surgery, The First Hospital of China Medical University, Shenyang 110001, China*
[b]*Key Laboratory of Precision Diagnosis and Treatment of Gastrointestinal Tumors, China Medical University, Ministry of Education, Shenyang 110001, China*
[c]*Phase I Clinical Trials Center, the First Hospital, China Medical University, Shenyang 110102, China*
[d]*Department of Urology, the First Hospital of China Medical University, Shenyang 110001, China*
[e]*Cancer Hospital of China Medical University, Liaoning Cancer Hospital & Institute, Shenyang 110001, China*
[f]*School of Pharmacy, China Medical University, Shenyang 110122, China*

*Corresponding authors.
E-mail addresses: bijianbin_cmu@163.com (Jianbin Bi), hongxuliu@qq.com (Hong-Xu Liu), liheranmm@163.com (Heran Li)

Received 21 January 2024
Received in revised form 24 January 2024
Accepted 25 January 2024