

Liposomes as nanocarriers for intracellular delivery of monoclonal antibodies for cancer treatment.

Natalia Majkowska^{1,2}, Alicja Hinz², Klaudia Gawron³, Joanna Lewandowska-Łańcucka³, Monika Bzowska²

¹Doctoral School of Exact and Natural Sciences, Jagiellonian University, Kraków, Poland

² Department of Cell Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Kraków, Poland

³Department of Physical Chemistry, Faculty of Chemistry, Jagiellonian University, Kraków, Poland

natalia.majkowska@doctoral.uj.edu.pl

Liposomes have gained significant attention as adaptable nanocarriers for targeted drug delivery. Their phospholipid bilayer structure mimics natural cell membranes, allowing for the protection of sensitive cargo and the potential for surface functionalization to enhance targeting and circulation time [1].

Monoclonal antibodies (mAbs) are a cornerstone of cancer immunotherapy, designed to bind specific antigens on tumor or immune cells. They can block signaling pathways, induce immune responses, or deliver cytotoxic agents. However, their therapeutic reach is limited to extracellular targets, as the mAb's large size and hydrophilic nature prevent them from entering cells and accessing intracellular oncogenic proteins that often drive malignancy.

Combining liposomal delivery systems with antibody-based therapies offers a potential strategy to overcome these limitations, enabling intracellular delivery while reducing systemic toxicity and off-target effects [2].

In this study, monoclonal antibodies targeting the c-MYC protein were successfully purified using affinity chromatography, yielding high-purity preparations suitable for encapsulation. Liposomes were separated from free antibodies using size-exclusion chromatography (SEC), which enabled efficient purification with minimal cross-contamination between liposomal and non-liposomal fractions.

The presence of antibodies inside liposomes was confirmed using multiple complementary techniques. ELISA was employed to detect encapsulated antibodies following liposome disruption. Additionally, fluorescently labeled liposomes and antibodies were analyzed using ImageStream flow cytometry, allowing for high-resolution visualization and quantification of co-localization within individual liposomal particles. These methods provided robust evidence of successful encapsulation and structural integrity of the liposomal formulations.

Furthermore, cellular uptake of liposomes containing labeled antibodies was demonstrated, confirming their potential as intracellular delivery vehicles.

These findings underscore the potential of liposome-based systems for the effective delivery of monoclonal antibodies. The successful confirmation of antibody encapsulation and cellular uptake highlights the functional integrity of the developed system. Additionally, the use of SEC allowed precise purification of liposomes, which is essential for downstream *in vitro* and *in vivo* applications.

Taken together, liposomal antibody delivery systems represent a potential therapeutic strategy that could significantly expand the scope of immunotherapy and contribute to the development of next-generation biologic drugs.

Acknowledgments

Studies were funded by NCN in the frame of the following project: OPUS25 UMO-2023/49/B/NZ6/02095

References

- [1] Tseu GYW, Kamaruzaman KA. A Review of Different Types of Liposomes and Their Advancements as a Form of Gene Therapy Treatment for Breast Cancer. *Molecules*. 2023 Feb 3;28(3):1498. doi: 10.3390/molecules28031498. PMID: 36771161; PMCID: PMC9920768.
- [2] M. Sela *et al.*, "Brain-Targeted Liposomes Loaded with Monoclonal Antibodies Reduce Alpha-Synuclein Aggregation and Improve Behavioral Symptoms in Parkinson's Disease," *Advanced Materials*, vol. 35, no. 51, Dec. 2023, doi: 10.1002/adma.202304654.

Figures

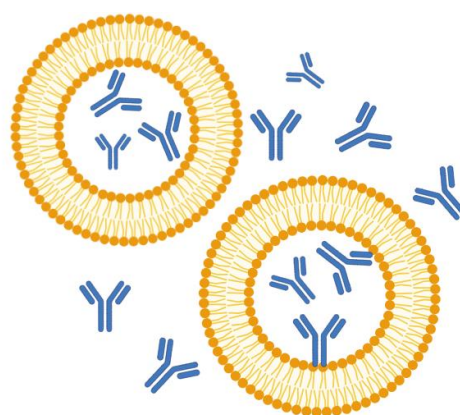


Figure 1. Scheme representing liposomes loaded with antibodies before purification. Figure prepared in BioRender.com