



Extracellular vesicles for improved intracellular accumulation of photosensitizers

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Cancer remains one of the most challenging medical problems worldwide, with high incidence and mortality. **Conventional therapies**, such as surgery, chemotherapy, and radiotherapy, often related with **limitations** including poor selectivity, systemic toxicity, and tumor resistance.

Photodynamic therapy (PDT) is a minimally invasive approach that uses photosensitizers (PS) to generate cytotoxic reactive oxygen species (ROS) upon light exposure. **The efficacy** of PDT depends on **efficient PS accumulation** in target cells and the preservation of PS photodynamic activity.

However, many PS exhibit **limited solubility and poor pharmacokinetics**, highlighting the need for effective delivery systems. **Extracellular vesicles (EVs)** have emerged as **promising nanocarriers**, offering biocompatibility, cargo protection, and efficient cellular uptake, thereby enhancing PDT selectivity and therapeutic potential.

Photodynamic therapy

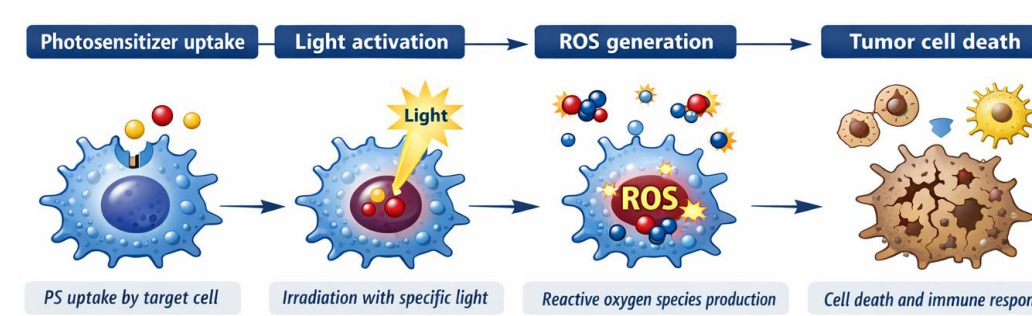


Fig. 1. Mechanism of photodynamic therapy (PDT) in cancer cells

EVs are nanosized structures enclosed by a lipid bilayer, secreted by cells into the extracellular space

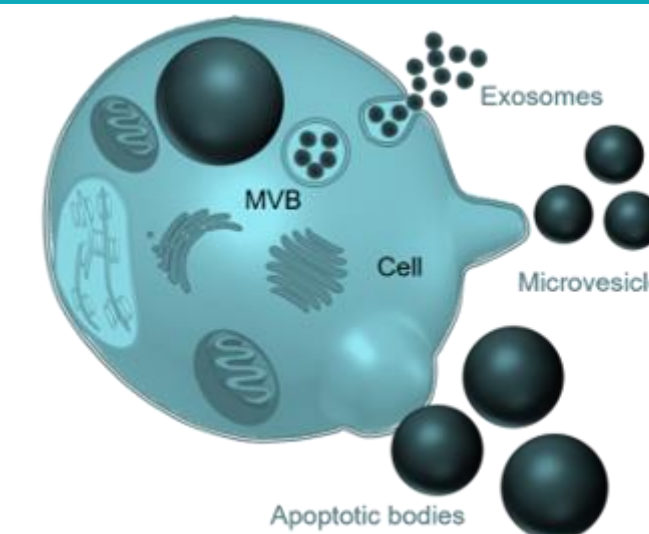


Fig. 2. Types of EVs secreted by different cells
DOI: 10.3390/cancers12020298

EXTRACELLULAR VESICLE ISOLATION

EVs were isolated from **HEK293 cells (human embryonic kidney cell)**

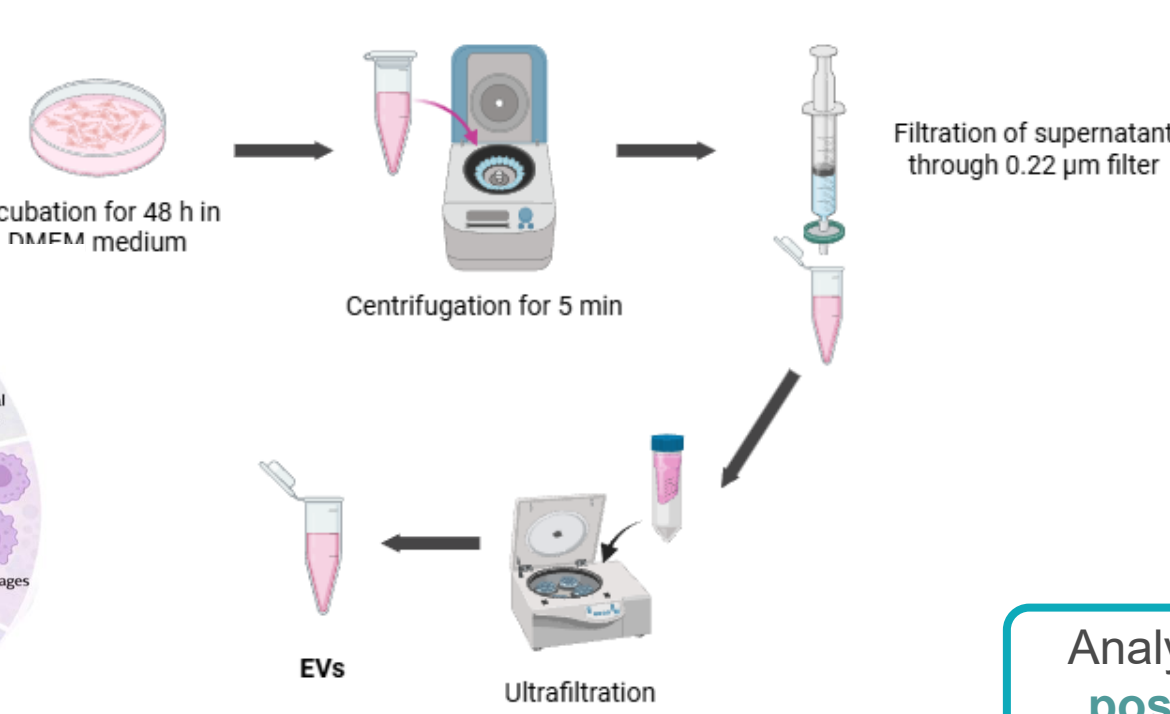


Fig. 4. Workflow for EVs isolation

Characterization of isolated EVs

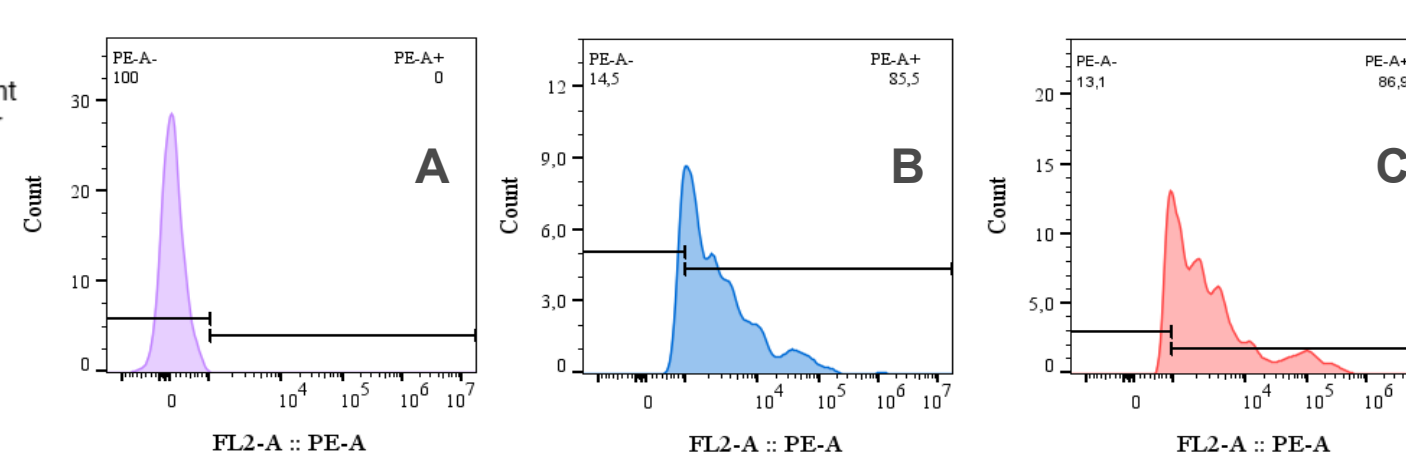


Fig. 5. Flow cytometry analysis: A – Control; B – CD9; C – CD63

Analysis confirmed that the **EV formulation** contains **CD9- and CD63-positive** vesicles with an average particle size of **289 nm** and a zeta potential of **-20 mV**

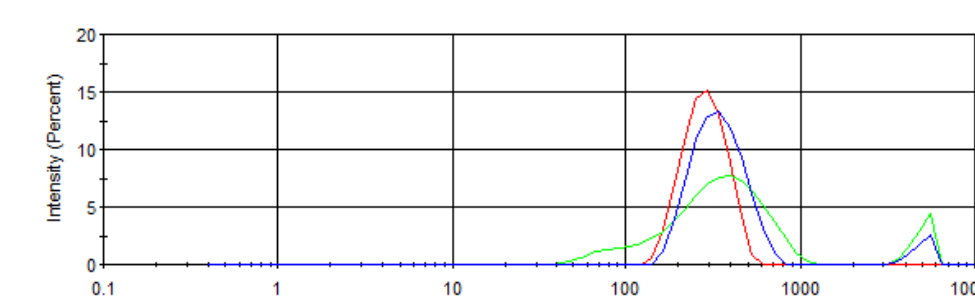


Fig. 6. Dynamic light scattering (DLS) analysis

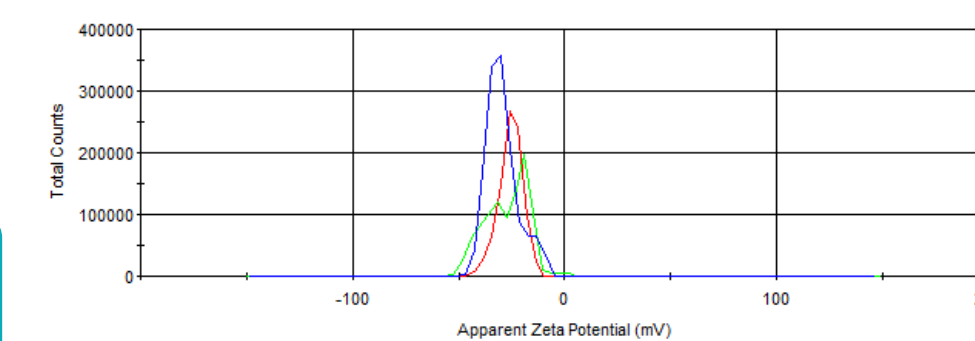


Fig. 7. Electrophoretic mobility (zeta potential) analysis

LOADING EVs WITH THE PHOTSENSITIZER (ZnP)

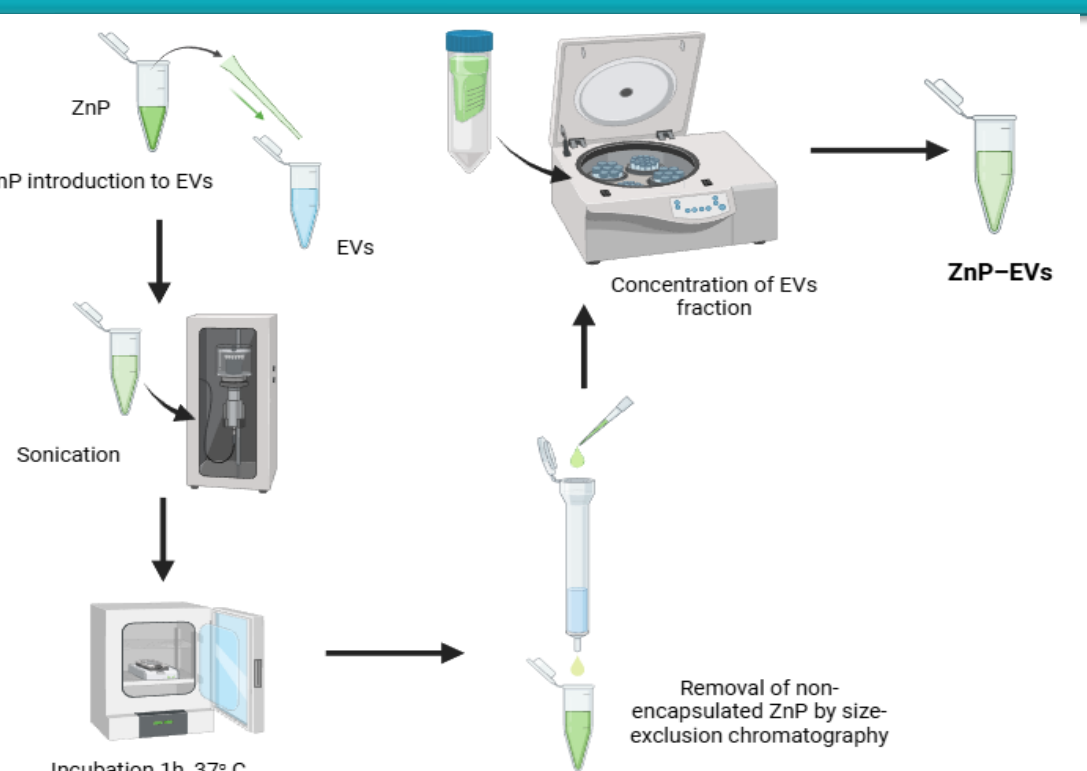


Fig. 8. Scheme of EV loading with photosensitizer (ZnP)

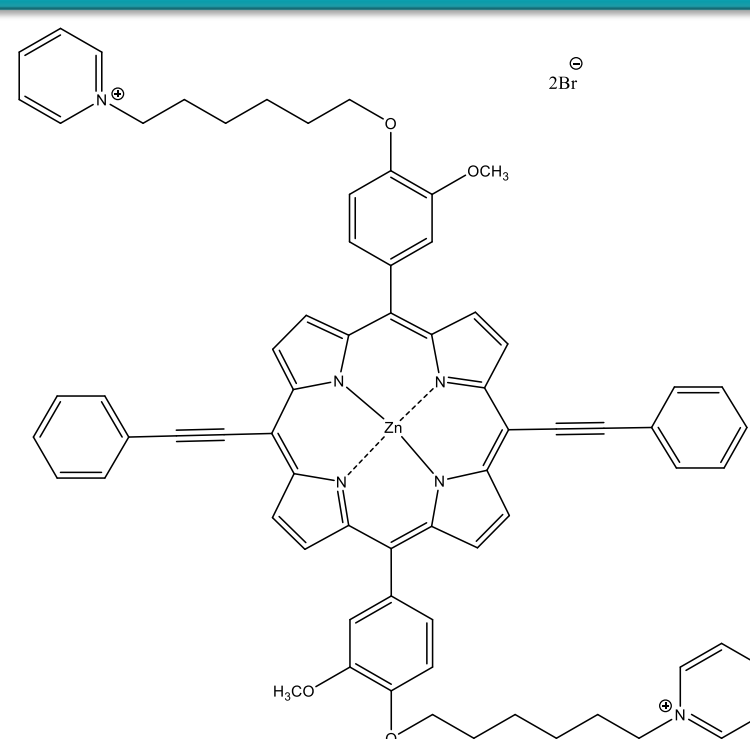


Fig. 9. Chemical structure of the photosensitizer **5,15-bis(3-methoxy(4-(6-pyridylhexyloxyphenyl)-10,20-di(ethynylphenyl)porphyrinato zinc (ZnP)**

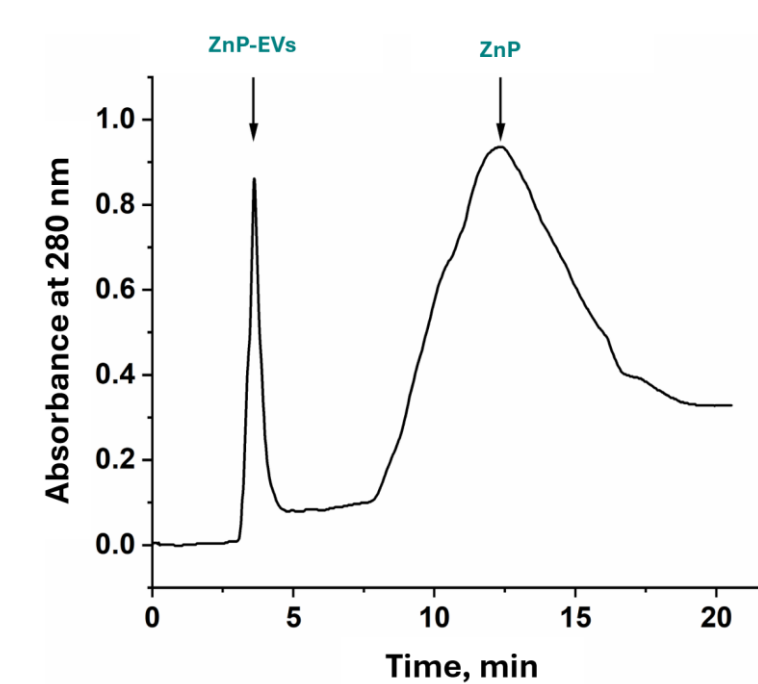


Fig. 10. Chromatogram of ZnP-loaded EVs after size-exclusion chromatography

FUNCTIONAL ACTIVITY ASSAYS

MTT assay

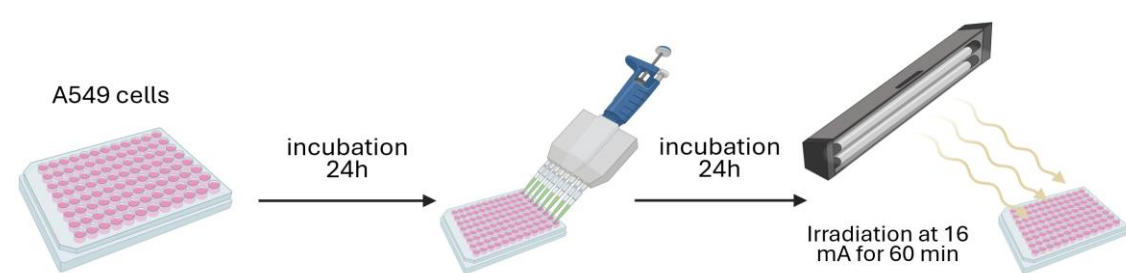


Fig. 11. Scheme of the cytotoxicity study of ZnP and ZnP-loaded EVs on A549 cells (human lung adenocarcinoma cells)

Results of MTT assay

Sample	IC50, µM
ZnP	0,10±0,01
ZnP-loaded EVs	0,42±0,04

ZnP encapsulation in EVs preserved the photodynamic activity of PS

Photodynamic ROS Generation

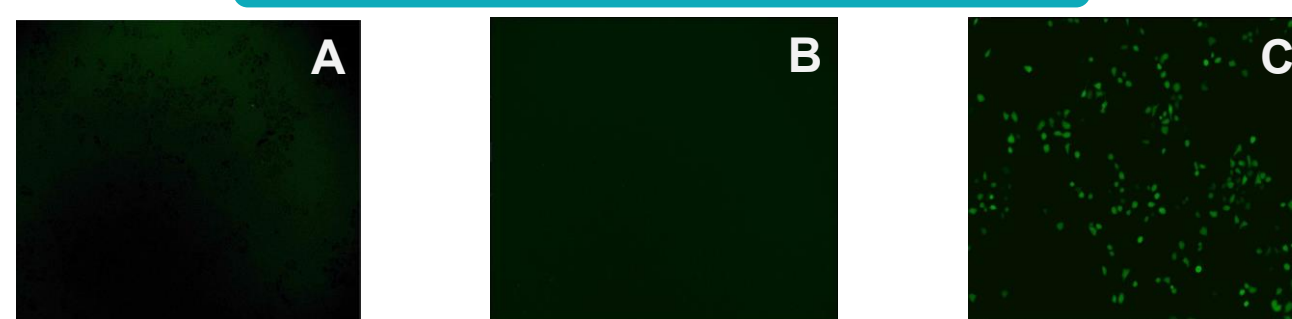


Fig. 12. Fluorescence microscopy of A549 cells incubated with samples: A – Control; B – ZnP; C – ZnP-EVs

ZnP-loaded EVs generate more reactive oxygen species than free ZnP, demonstrating enhanced photodynamic activity

ZnP release from EVs

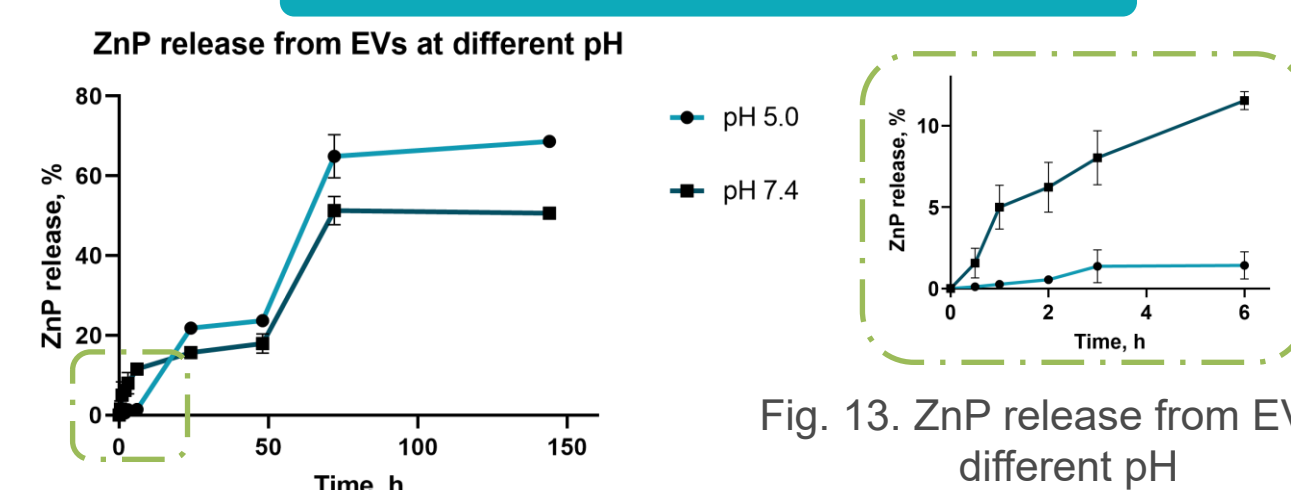


Fig. 13. ZnP release from EVs at different pH

ZnP is released from EVs in a controlled manner, with 22% released at pH 5.0 and 16% at pH 7.4 within 24 hours

CONCLUSION

- HEK293-derived EVs obtained in this study had an average size of **289 nm** and a zeta potential of **-20mV**, confirming their **nanoscale** dimensions and membrane **stability**
- The ZnP content in EVs suspension was **~6 µg/mL**, confirming **successful** development of ZnP delivery system
- ZnP release profile from EVs demonstrated a biphasic pattern; within 24 h, cumulative ZnP **release** was **22%** at pH 5.0 and **16%** at pH 7.4, indicating controlled, pH-responsive release
- MTT assay showed **the absence of ZnP-EVs dark toxicity** and preserved **photodynamic activity** in A549 cells upon irradiation
- Fluorescence microscopy analysis demonstrated that ZnP-loaded EVs **generated ROS more efficiently** than free ZnP

The results demonstrate that EVs derived from HEK293 cells preserve the photodynamic properties of the encapsulated ZnP, enable controlled release under biologically relevant conditions and enhance ROS generation compared to free ZnP