Polymer-lipid hybrid nanoparticles improve drug-resistant lung cancer treatment

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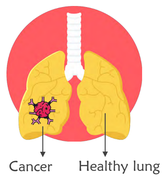
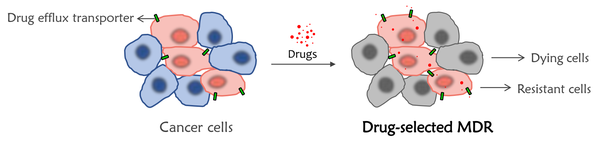
Introduction

Lung cancer is one of the most common cancers and the main causes of cancer mortality in the world.

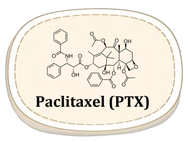


Multidrug resistance (MDR) and metastasis or the spread of cancer cells are major causes leading to failure of cancer treatment. **MDR** can develop in two main ways, with differences in their mechanisms for drug resistance.

1 **Drug-selected MDR** developed after chemotherapeutic treatment by increase the activity of drug efflux pump.



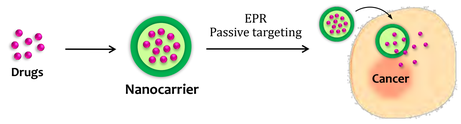
2 **Metastasis-associated MDR** acquired by cellular adaptation to microenvironmental changes during metastasis.



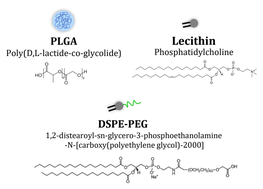
Clinically effective against various cancers

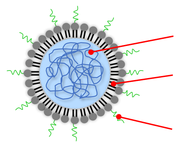
Low water solubility affects its bioavailability

Anti-cancer drugs should be encapsulated into nanocarriers to improve its solubility and allow the stable dispersion in aqueous systems.



Delivery system





Polymer-lipid hybrid nanoparticles

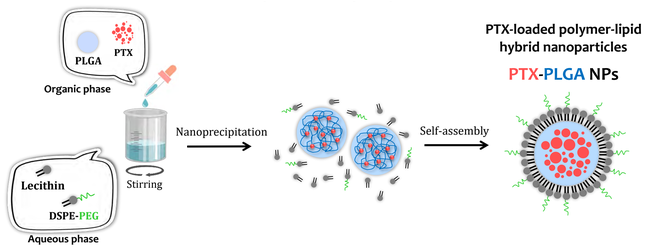
(i) A **hydrophobic polymeric core** where poorly water-soluble drugs are incorporated with high loading yields
(ii) A **lipid layer surrounding the core** that acts as a highly biocompatible shell and as a molecular fence to promote drug retention inside the polymeric core
(iii) A **hydrophilic polymer stealth layer (PEG) outside** the lipid shell to enhance nanoparticle stability and systemic circulation lifetime

Aim

To develop polymer-lipid hybrid nanoparticles for delivery of anticancer drug, paclitaxel (PTX) to overcome multidrug resistance (MDR) of human lung cancer cells.

Methods & Results

Nanoparticle preparation



Nanoparticle characterization

Nanoparticles were analyzed in terms of particle size and polydispersity index (PDI) by Dynamic Light Scattering (DLS) and the zeta potential was determined via electrophoretic mobility using Zetasizer Nano ZS90 (Malvern instruments, UK).

Nanoparticle preparation : Effect of organic solvent

Table 1 Characterization of polymer-lipid nanoparticles prepared in different organic solvent.

	Organic solvent	Size	PDI	Zeta potential
PLGA NPs	Acetronitrile	94.6 ± 2.7 nm	0.09	-48.6 ± 1.9 mV
	Tetrahydrofuran	128.3 ± 4.2 nm	0.04	-36.7 ± 4.7 mV

MORE POLAR SOLVENT → MORE WATER MISCIBLE → LOWER PARTICLE SIZE

PTX-PLGA NPs preparation using Acetronitrile as organic solvent

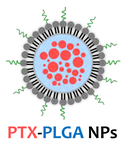


Table 2 Characterization of PTX-loaded polymer-lipid nanoparticles.

	Size	PDI	Zeta potential
PLGA NPs	94.6 ± 2.7 nm	0.09	-48.6 ± 1.9 mV
PTX-PLGA NPs	103.0 ± 1.6 nm	0.11	-52.9 ± 2.1 mV

PTX-PLGA NPs had an average size of 103.0 nm, which larger than those nonencapsulated. Nanoparticles exhibited uniform size with polydispersity index (PDI) of < 0.15. The negative zeta potential or surface charge is due to the presence of carboxyl end groups of lipid-PEG at the particle surface. Particles with zeta potentials more negative than -30 mV are normally considered stable in solution.

Methods & Results

Drug-selected MDR

A549RT-eto cells
MDR cell line derived from culturing of A549 cells under adherent monolayer culture condition with continuous exposure to anticancer drug Etoposide.

Metastasis-associated MDR

A549 floating cells
Detachment-induced MDR acquired by culturing A549 cells without drug pretreatment under non-adherent conditions in polyHEMA-coated plates, which mimic metastasizing cells in the blood/lymphatic circulations.

Protein expression analysis
Expression of the proteins of interest in cell lysates were detected by immunoblot technique.

Gene expression analysis
Expression of target gene was determined by qRT-PCR technique. The $2^{-\Delta\Delta C_P}$ method was used to calculate fold change in target gene expression by comparing relative expression of A549RT-eto and A549 floating cells with A549 attached cells using β-Actin as a reference gene.

Cytotoxicity assay
Sensitivity of cells to chemotherapeutic drugs was evaluated by MTT assay for the adherent condition and modified MTT assay for the non-adherent condition.

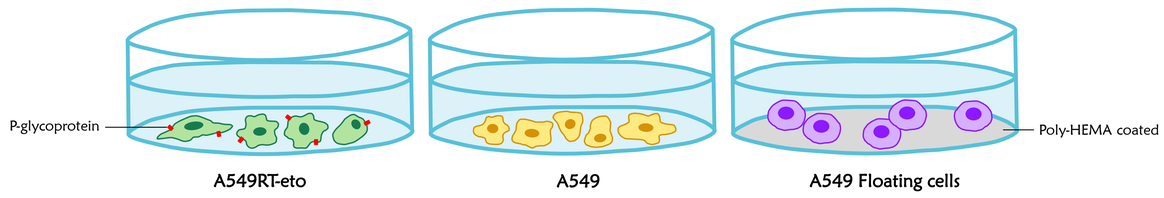


Figure 1 Schematic diagram of A549, A549RT-eto and A549 floating cells

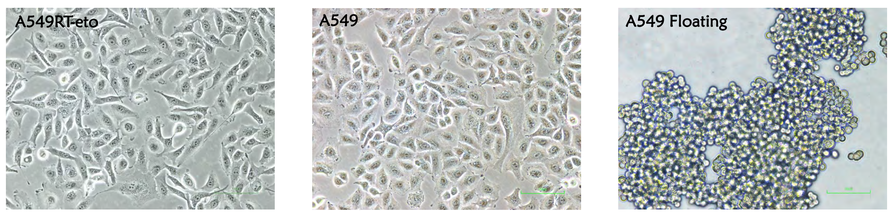


Figure 2 Morphology of A549, A549RT-eto and A549 floating cells

Cytotoxicity of PTX and PTX-PLGA NPs

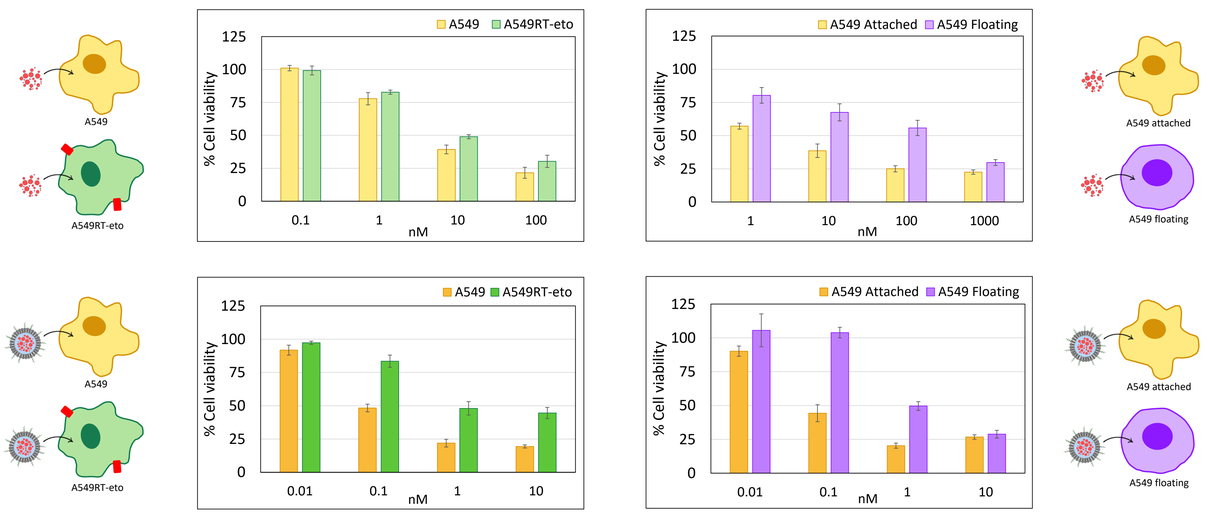


Table 3 IC_{50} values of drugs in A549 and A549RT-eto cells.

Drugs	IC_{50} at 72 h	
	A549	A549RT-eto
PTX	5.33 ± 0.91 nM	9.50 ± 0.87 nM
PTX-PLGA NPs	0.11 ± 0.03 nM	0.93 ± 0.11 nM
Fold change in IC_{50}	49	10

Table 4 IC_{50} values of drugs in A549 attached and A549 floating cells.

Drugs	IC_{50} at 72 h	
	A549 Attached	A549 Floating
PTX	4.70 ± 1.45 nM	289.01 ± 155.82 nM
PTX-PLGA NPs	0.11 ± 0.45nM	1.13 ± 0.21 nM
Fold change in IC_{50}	43	256

The IC_{50} values were decreased by 49-fold and 10-fold in A549 and A549RT-eto cells treated with nanoparticles, respectively. The IC_{50} values of A549 attached and A549 floating cells treated with nanoparticles were decreased by 43-fold and 256-fold, respectively.

Conclusions

Nanoparticles presented superior cytotoxicity by increases PTX potency as indicated by the decrease in IC_{50} values compared to non-encapsulated PTX. These findings indicated that the PTX-loaded polymer-lipid hybrid nanoparticles provide a promising delivery system for treatment of drug resistant lung cancer.

Acknowledgements

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References

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