Novel Pseudomonas putida-derived nanoliposomes: Physico-chemical properties and Anticancer effect

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Introduction

Pseudomonas putida (PP) is a gram-negative and rod-shaped bacterium that is generally isolated from soil [1]. In this study, we aimed to utilize phospholipids from the bacterium Pseudomonas putida (PP) as a plentiful, safe, and accessible resource for creating nanoliposomes to deliver doxorubicin (Dox) to MCF-7 breast cancer cells. This bacterium provides a cost-effective source of phospholipids commonly used in nanoliposome production, with no toxicity or adverse environmental impact. To this end, molecular dynamics (MD) simulations were first conducted to evaluate the feasibility of this approach and to analyze the behavior and interaction of Dox with the nanoliposomes [2].

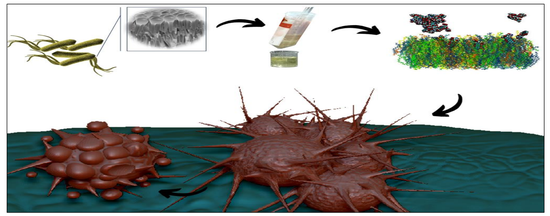
After evaluating the physicochemical properties, we did an in vitro study to investigate the anti-cancer properties of this nano drug delivery system on the breast cancer cell line, MCF-7.

Methods

MD simulations were performed to evaluate the interactions between the membrane phospholipids of the synthesized carrier and Dox. The phospholipids of PP were extracted according to Folch method [35] with minor modifications. . Subsequently, Dox-loaded PP-derived nanoliposomes (PNL-Dox) were developed using the thin-film method. The physicochemical properties of the fabricated nanocarrier such as the mean particle size (Z-Average), polydispersity index (PDI), zeta potential, Fourier transform infrared spectroscopy (ATR-FTIR), in vitro release profile, scanning electron microscope (SEM) , Entrapment efficiency (EE) and Drug loading (DL) of PNL-Dox and PNL-B were investigated and the anticancer effects of this system such as Cytotoxicity assay; To determine the cytotoxicity of Dox, PNL-B and PNL-Dox against MCF-7 cells, an MTT assay was performed [40], Intracellular uptake; to assess the ability of PNL-Dox's cellular uptake in MCF-7 cells, the nanoliposomes were labeled with fluorescein isothiocyanate (FITC), DAPI staining, Apoptosis; FITC-conjugated Annexin V/propidium iodide (PI) co-staining was performed to assess the type of cell death, and the ROS level was measured using the fluorogenic 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA) probe. were tested on MCF-7 cells.

Results

The results of the MD simulations indicated that Dox reacted with all of the phospholipids through hydrogen bonds without affecting the fluidity, stability, and thickness of the nanoliposome membrane. Additionally, a small number of Dox molecules interacted with the nanocarrier membrane, while the remaining were located in its interior. The physicochemical investigation results showed that PNL-Dox had an average particle size and zeta potential of 271.7 ± 7.1 nm and -8.8 ± 3.3 mV, respectively. Scanning electron microscopy revealed that the particles were spherical and did not show any signs of aggregation. Drug release of PNL-Dox was gradual at pH 7.4 and 6.5, with a significantly higher release at pH 6.5. In vitro studies demonstrated successful uptake of PNL-Dox by MCF-7 cells, resulting in cytotoxicity within 24 and 48 hours of treatment. Also, it increased apoptosis and reduced the production of reactive oxygen species (ROS) in these cells. Our study showcased the potential of PP phospholipids to form a promising anti-cancer drug delivery system, opening up new possibilities for the treatment of all types of cancers.



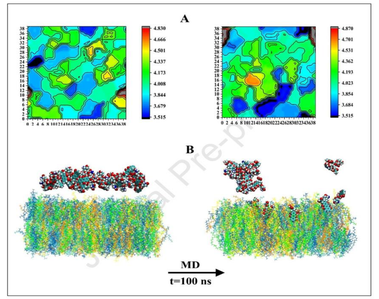


Figure 4: Membrane physical characteristics before and after interaction with Dox. A) 2D thickness contour of the membrane of a) before, and b) after interaction with Dox respectively from 100 ns MD simulations. B) Schematic representation of the location of Dox molecules from 100 ns MD simulations

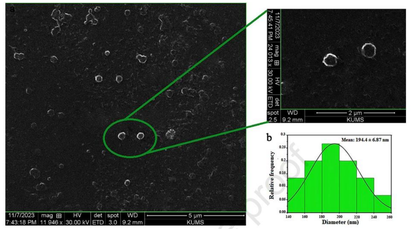


Figure 5: a) Scanning electron microscopy (SEM) of PNL-Dox in two scales (5 and 2 µm) revealed the spherical shapes of the nanoliposome with no aggregation. b) Particle size distribution of PNL-Dox showed the mean particle size of 194 ± 6.87 nm.

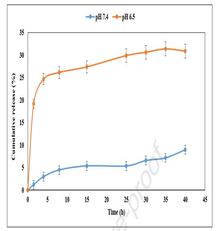


Figure 7: In vitro pH-responsive drug release of the PNL-Dox in different pH values to simulate the physiological (pH 7.4) and tumor (pH 6.5) conditions. At pH 7.4, the release of Dox was lower compared to the release at acidic pH at each time point.

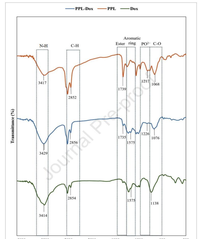


Figure 6: FTIR spectra of Dox, PNL-Dox and bacterial phospholipids (PPL) at the wavelength range of 4000-400 cm⁻¹. The peaks confirm the successful synthesis of PNL-Dox.

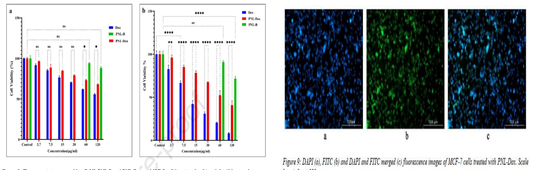


*Figure 8: The cytotoxicity assay of free DOX, PNL-B and PNL-Dox on MCF-7 cell line (a) after 24 and (b) 48 hours of treatment. The values were expressed as mean ± SD of three independent experiments. Ns, not significant, *p < 0.05, **p < 0.01, ***p < 0.001.*

Figure 9: DAPI (a), FITC (b) and DAPI and FITC merged (c) fluorescence images of MCF-7 cells treated with PNL-Dox. Scale bars indicate 200 µm.

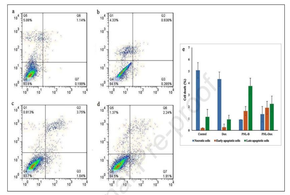


Figure 10: Annexin V-FITC/PI co-staining of MCF-7 cells to evaluate apoptosis. (a) control cells; (b) cells treated with Dox; (c) cells treated with PNL-B; (d) cells treated with PNL-Dox. Q1, Q2, Q3 and Q4 showed necrotic, late apoptotic, early apoptotic and live cells respectively. (e) Histogram of apoptosis of MCF-7 cells after 24 hours of treatment with Dox, PNL-B and PNL-Dox.

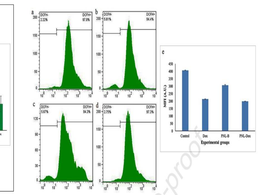


Figure 11: The ROS production in MCF-7 cells after 24 hours of treatment with (b) Dox; (c) PNL-B; (d) PNL-Dox. (a) represents untreated control cells. The fluorescence intensity is expressed as MFI. The MFI values were 496.5 ± 1.5, 324 ± 1, 307 ± 4 and 109 ± 1 for Control, Dox, PNL-B, and PNL-Dox treated groups respectively.

Conclusions

In this study, we developed novel nanoliposomes using PP-extracted phospholipids to load and deliver Dox to MCF-7 breast cancer cells. MD simulation demonstrated that loading Dox in this nanocarrier was energetically favorable and did not negatively impact its stability, fluidity, or thickness. Our results also showed that this system had suitable physicochemical properties with a size of 271.7 ± 7.1 and a zeta potential of -8.8 ± 3.3. Moreover, PNL-Dox showed a pH-responsive release profile, enabling the gradual release of Dox in a target-specific manner especially at pH 6.5. Treatment with this system resulted in decreased viability of MCF-7 cells with a half-maximum inhibitory concentration of 60 µg/ml. PNL-Dox was also found to reduce production of ROS in this concentration. Exposure to PNL-Dox led to an increase in late apoptosis. In summary, presents a promising new nanocarrier for the treatment of various types of cancer.