

Docetaxel-loaded solid lipid nanoparticles prevent tumor growth and lung metastasis of 4T1 murine mammary carcinoma cells

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- **Nanoparticle:** SLN-DTX (**Fig. 1a**)
 - SLN = solid lipidic nanoparticles
 - * Lipidic matrix = Compritol 888 ATO
 - * Surfactants = Pluronic F127 + Span 80
 - DTX = docetaxel (chemotherapeutic agent, **Fig. 1b**)
- **Preparation:** high-energy method
- **Characterization:**
 - DLS
 - * zeta potential (ζ)
 - * hydrodynamic diameter (HD)
 - * polydispersity index (PDI)
 - Stability
 - * time (**Fig. 2a**)
 - * storage (varying temperatures, **Fig. 2b**)
 - * biological media (water, PBS, cell media, and serum)
 - SLN-DTX morphology and mean size (TEM, **Fig. 2c**)
 - Drug release (**Fig. 2d**)
 - * pH 7.4 (physiological environment)
 - * pH 5.0 (acidic tumor cell intracellular environment)
 - Presence/absence of DTX in SLN-DTX
 - * FTIR (**Fig. 3a**)
 - * Raman (**Fig. 3b**)
 - * DSC (**Fig. 3c, Table 1**)
 - Cytotoxicity
 - * MTT: 24 h and 48 h incubation
 - * Cancer cells: 4T1 (**Fig. 4a, b**) and MCF7 (**Fig. 5a, b**)
 - * Healthy cells: NIH-3T3 (**Fig. 4c, d**) and HNTMCs (**Fig. 5c, d**)

- Cell morphology (healthy and 4T1, before and after exposure to DTX or SLN-DTX)
 - * Light (phase contrast) microscopy (**Fig. 6a**)
 - * SEM (**Fig. 6b**)
- Cell cycle arrest (G2/M phase, **Fig. 7a**)
- Number of apoptotic cells (Annexin V | propidium iodide, **Fig. 7b**)
 - * viable: – | –
 - * necrosis: – | +
 - * early apoptosis: + | –
 - * late apoptosis: + | +
- Cell morphology (4T1 cells, untreated or treated with DTX or SLN-DTX)
 - * Immunofluorescence staining (**Fig. 8**)
 - * tubulin (cytoskeleton)
 - * DAPI (nucleus)
- In vitro uptake
 - * Internalization and ultrastructure features (TEM, **Fig. 9a**)
 - * HPLC method for quantifying the amount of DTX uptaken by the cells (**Fig. 9b**)
- Entrapment efficiency (EE%)

$$\text{Entrapment efficiency (EE\%)} = \frac{\text{mass of DTX in SLNs}}{\text{mass of DTX used in SLNs preparation}} \times 100$$

- Drug loading (DL%)

$$\text{Drug loading (DL\%)} = \frac{\text{mass of DTX in SLNs}}{\text{mass of SLNs}} \times 100$$

- In vivo assays (female Balb/c mice with subcutaneously induced 4T1 solid tumors)
 - * 5 groups (healthy, PBS, DTX, SLN, and SLN-DTX)
 - * 5 doses (1 every 4 days)
 - * Tumor growth over time (**Fig. 10a**)
 - * Animal mass (index of systemic toxicity, **Fig. 10b**)
 - * Hematological and biochemical assays
 - * Lung metastasis (number of visible nodules + histopathology, **Fig. 11a-c**)
 - * IL-6 expression: pro-inflammatory cytokine, biomarker for cancer in development, progress, and metastasis (**Fig. 11d**)
 - * Ki-67 expression: cell cycle regulatory protein, biomarker for cell proliferation (lungs, **Fig. 12a-c**; tumor: **Fig. 12d-f**)
 - * Bcl-2 expression: protein encoded by *Bcl-2* protooncogene, biomarker for advanced metastatic cancer (lungs, **Fig. 12a-c**; tumor: **Fig. 12d-f**)