Nano 2024 Innovation Conference & Exhibition — Rome, 9-13 September

Doxorubicin-loaded super stealth liposomes as advanced nanomedicine for the treatment of metastatic breast cancer

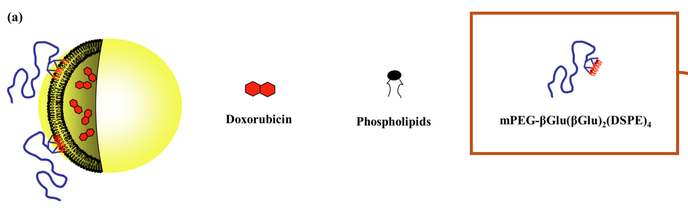
Luigi Ciriolo[1], Donatella Paolino [2,3], Nicola d'Avanzo [2,3], Elena Canato [4], Antonella Grigoletto [4], Maria Chiara Cristiano[5], Antonia Mancuso [2,3], Christian Celia [6], Gianfranco Pasut [4] and Massimo Fresta [1]

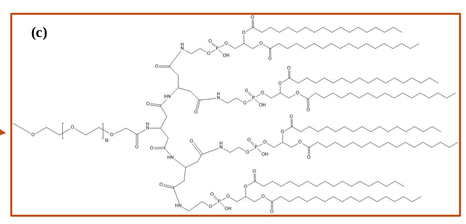
[1] Department of Health Science, University of Catanzaro "Magna Græcia", V.le "S. Venuta", Catanzaro, 88100, Italy
[2] Department of Clinical and Experimental Medicine, University of Catanzaro "Magna Græcia", V.le "S. Venuta", Catanzaro, 88100, Italy
[3] Research Center "ProHealth Translational Hub", Department of Experimental and Clinical Medicine, "Magna Graecia" University of Catanzaro, V.le "S. Venuta", 88100 Catanzaro, Italy
[4] Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Via F. Marzolo 5, 35131 Padua, Italy.
[5] Department of Medical and Surgical Sciences, University "Magna Græcia" of Catanzaro, V.le "S. Venuta", Catanzaro, Italy
[6] Department of Pharmacy, University of Chieti – Pescara "G. d'Annunzio", Via dei Vestini 31, 66100, Chieti, Italy.

luigi.ciriolo@studenti.unicz.it; fresta@unicz.it

PEGylated liposomes have been used in clinic for 30 years as chemotherapeutic drugs' delivery system. Polyethylene glycol (PEG) can increase the half-life of nanosystems reducing the opsonization phenomena that usually occur in bloodstream. However, commercial PEGylated phospholipids did not show proper stability within the bilayer, causing the reduction of the PEG-coating on the surface of nanomedicine after systemic injection. To overcome this drawback, we developed synthetic PEGylated dendron phospholipids ensuring an improved anchoring effect to the liposomes' bilayer for increasing the long-circulation properties of resulting super stealth liposomes (SSLs). SSLs showed suitable physicochemical properties for in vivo administration of Doxorubicin (Dox) and significantly increased the efficacy of Dox in tumor-bearing mice compared to free drug. Dox-loaded SSLs (SSLs-Dox) also provided a depot system, ensuring the accumulation of drug in the tumor site even after stopping the treatment, thus allowing to improve the survival rate. Therefore, SSLs have the similar biopharmaceutical properties of conventional stealth liposomes, even showing a significantly improved long-circulation time after systemic injection. The obtained results suggest that doxorubicin-loaded super stealth liposomes may represent an effective nanomedicine for the treatment of metastatic breast cancer.

Physicochemical characterization





(b)

	Liposomes			
	Size (nm)	PDI	Z-potential (mV)	E.E. (%)[2]
empty-SSLs	164.0 ± 3.0	0.12 ± 0.03	-40.5 ± 1.2	–
SSLs-Dox	189.3 ± 3.5**	0.21 ± 0.01**	-38.6 ± 0.6	88.0 ± 3.0

[1]PDI: Polydisperity index; [2]E.E. (%): entrapment efficiency percentage

Figure 1. Physicochemical properties of empty-SSLs and Dox-SSLs. Panels (a) and (c) are schematic representations of SSLs and synthetized PEG-dendron phospholipid, respectively. Dynamic light scattering (DLS) analysis was carried out at 25 ± 1 °C (b). Results represent the average of five independent experiments ± standard deviation. Statistical significance: *p <0.05 and **p <0.01.

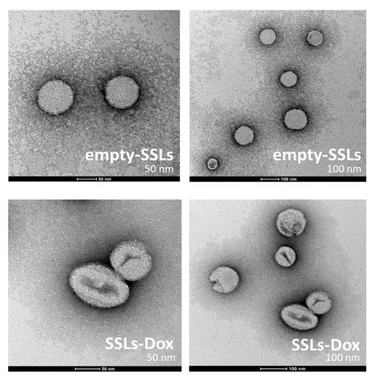


Figure 2. TEM characterization of empty-SSLs and Dox-SSLs.

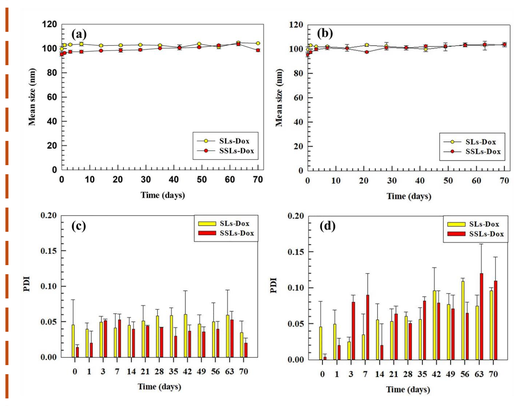


Figure 3. Stability of Dox-loaded conventional stealth liposomes (SLs-Dox) and SSLs-Dox in PBS solution at 4 and 25 °C up to 70 days. Panels (a) and (b) show the mean size of SLs-Dox and SSLs-Dox at 4 °C and 25 °C, respectively; while panels (c) and (d) show the PDI values at 4 °C and 25 °C, respectively.

In vitro tests

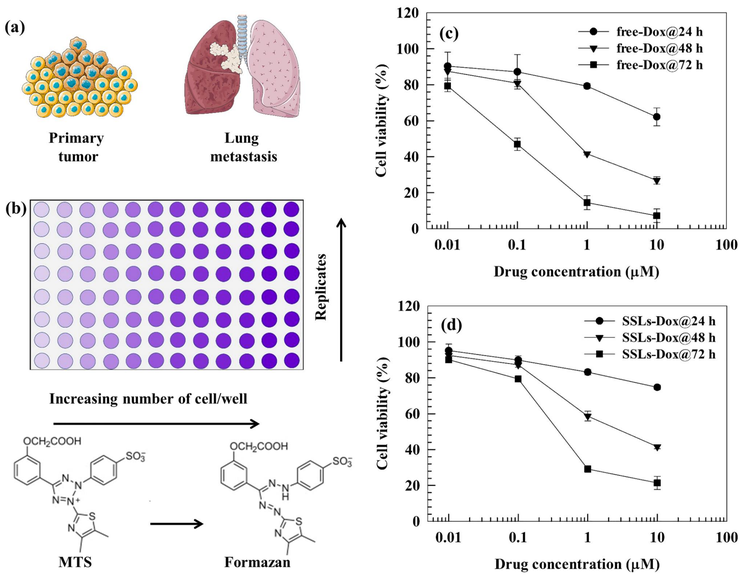


Figure 4. In vitro viability assay. Panels (a) and (b) are schematic representations of the primary/metastatic breast cancer and MTS test, respectively, while panels (c) and (d) show the cytotoxic effects of free-Dox and SSLs-Dox on the MDA-MB-231 cell line. MDA-MB-231 cells (5000 cells per well) were seeded into a 96-well plate. Free-Dox or SSLs-Dox, were added at different concentrations (0.01, 0.1, 1, and 10 μM), and the cells were then incubated at 37 °C for 24, 48, and 72 h. The error bars, if not shown, are within the symbols.

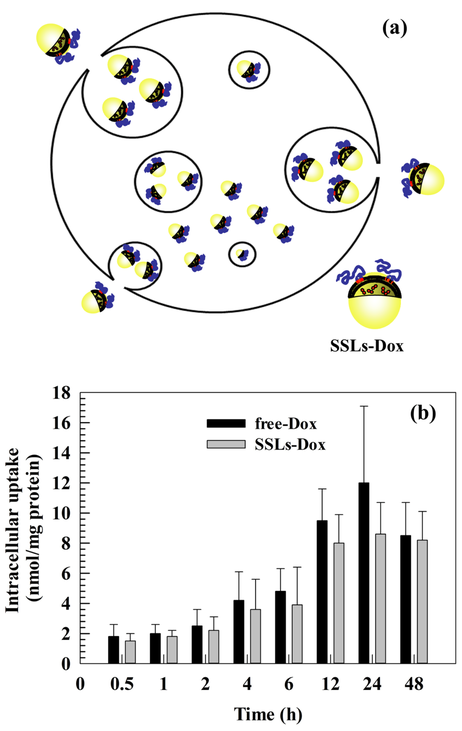
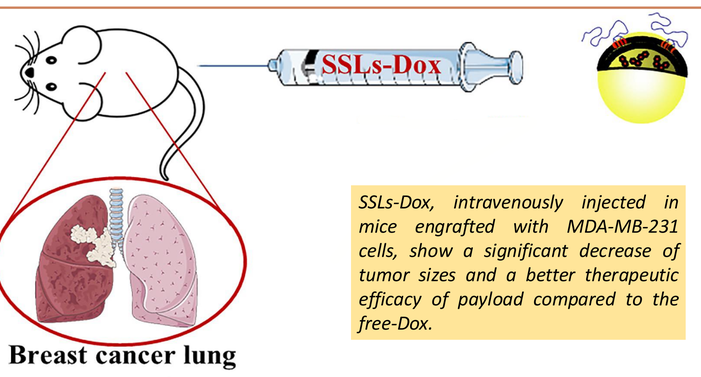


Figure 5. Intracellular uptake of free-Dox and SSLs-Dox in MDA-MB-231 cells. Panel (a) show the mechanism of intracellular uptake of SSLs into cancer cells. Cells were incubated with free-Dox and SSLs-Dox up to 48 h (b). Results are the average of three different experiments ±S.E. Each point is collected from 6 wells. The error bars, if not shown, are within the symbols.



SSLs-Dox, intravenously injected in mice engrafted with MDA-MB-231 cells, show a significant decrease of tumor sizes and a better therapeutic efficacy of payload compared to the free-Dox.

Pharmacokinetic studies

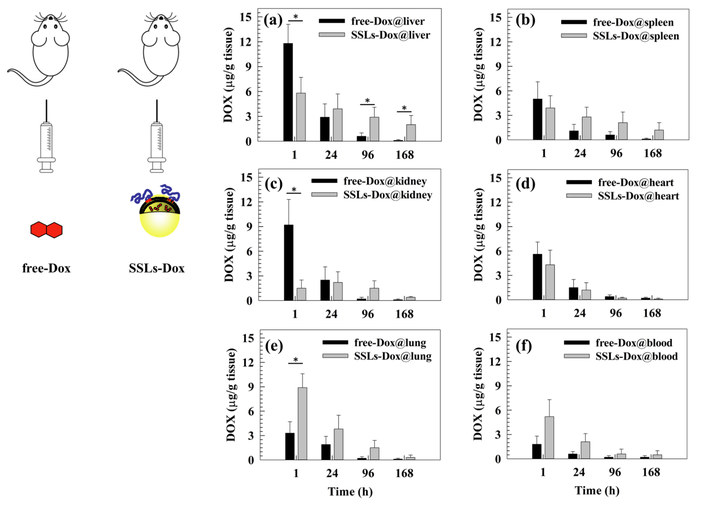


Figure 6. Doxorubicin hydrochloride biodistribution. The accumulation of Dox in the liver (a), spleen (b), kidney (c), heart (d), lung (e), and blood (f) of MDA-MB-231 tumor-bearing mice treated by free-Dox and SSLs-Dox. Mice were sacrificed at different time points, i.e., 1 h, 24 h, 4 days, and 7 days. The Dox was extracted from the tissues or blood and measured by HPLC. *p <0.05, **p <0.01 represent statistically significant differences of free-Dox versus SSLs-Dox.

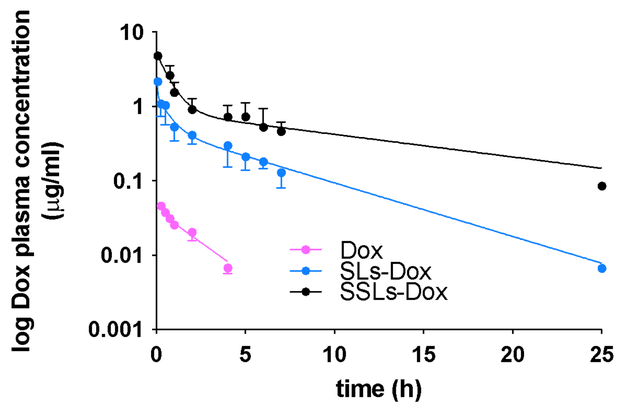


Figure 7. Pharmacokinetic experiments. Pharmacokinetic profiles of Dox, SLs-Dox, and SSLs-Dox were studied in rats (n = 3) after the intravenous (i.v.) injection of 2.5 mg/kg of Dox equivalent drug dosage per animal.

In vivo studies

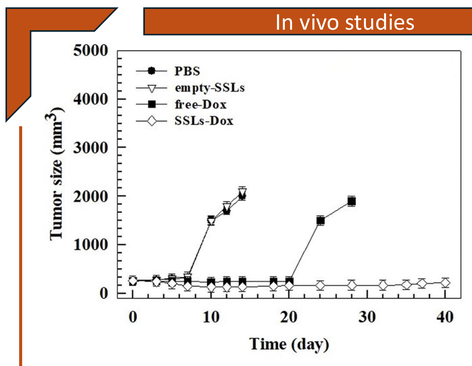


Figure 8. Monitoring of tumor size after treatment. The injected dose of SSLs-Dox was 3 mg/kg, equivalent to free-Dox dosage for each administration. PBS and empty SSLs were used as control. Tumor volumes of different groups were measured with a caliper. At day 14 and day 28, the mice of PBS/empty-SSLs and free-Dox groups were sacrificed because the tumor volume exceeded 2000 mm³.

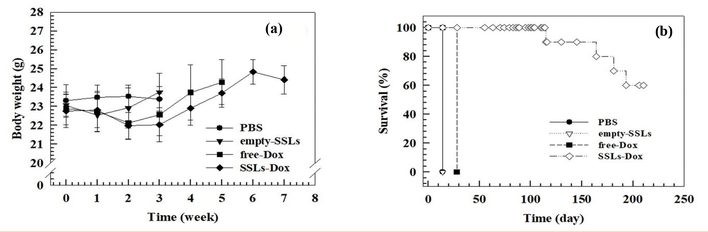


Figure 9. Monitoring of health conditions after treatment. (a) Changes in body weight were monitored weekly. No significant differences between these four groups were found, but a drop in body weight was obtained for the groups after the first injection. (b) Survival rate of tumor-bearing mice was studied for up to 210 days (*n* = 10 per group). The survival rate tests of the PBS, empty-SSLs, and free-Dox groups were discontinued due to the overgrowth of the tumor size (volume >2000 mm³).

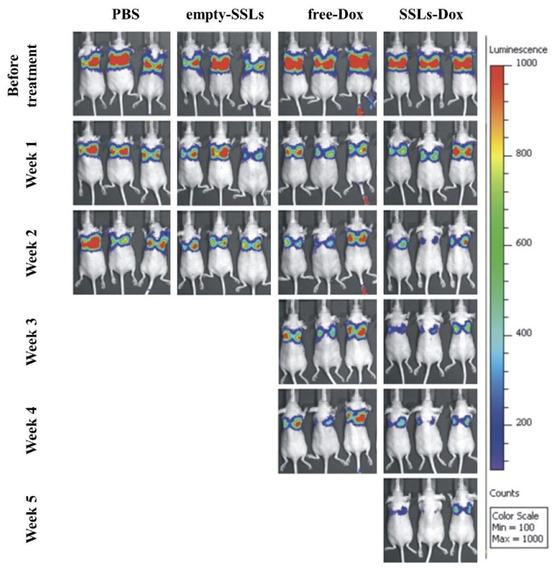


Figure 10. *In vivo* bioluminescence of tumor-bearing mice. *In vivo* bioluminescence of tumor development in mice treated with PBS, empty-SSLs, free-Dox, and SSLs-Dox. PBS and empty-SSLs were discontinued in the studies at the end of week 2, while free-Dox group was withdrawn at the end of week 4 in accordance with the animal welfare guidelines (Tumor volume >2000 mm³).

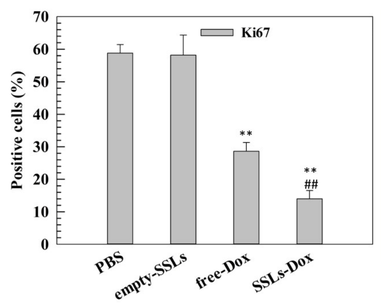


Figure 11. Ki-67 staining test. The Ki-67 positive staining of lung cells in metastatic tissues was carried out in the different tested groups: PBS, empty-SSLs, free-Dox, and SSLs-Dox. Ki67-positive cell index was calculated by counting Ki67-positive cells for PBS, empty-SSLs, free-Dox, and SSLs-Dox. Bars represent the mean ± S.D. Statistical significance: *p <0.05 and **p <0.01 (free-Dox and SSLs-Dox *versus* PBS); #p< 0.05 and ##p< 0.01: (free-Dox *versus* SSLs-Dox).

References

Paolino, D., d'Avanzo, N., Canato, E., Ciriolo, L., Grigoletto, A., Cristiano, M. C., Mancuso, A., Celia, C., Pasut, G. & Fresta, M. (2024). Improved Anti-breast Cancer Activity by Doxorubicin-loaded Super Stealth Liposomes. Biomaterials Science.

SCAN ME