TROP-2 TARGETING IMMUNOLIPOSOME FORMULATION AS METFORMIN DRUG DELIVERY SYSTEM

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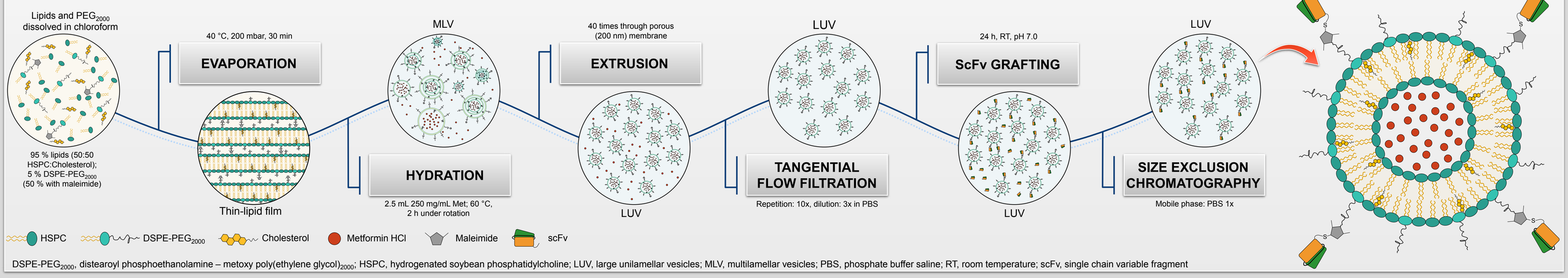
INTRODUCTION

This study focuses on the optimisation of metabolic modulator metformin (Met) loaded immunoliposome formulation. This drug, commonly used to treat type II diabetes, has recently shown to have promising properties in the context of cancer treatment[1,2]. However, as an orally taken drug, it shows low bioavailability, and rapid renal clearance making them ineffectual[3], which is why nanovectorisation is needed. Due to their large aqueous compartment, and flexible formulation liposomes were used as Met delivering vesicles. Optimal thin-film hydration conditions of Met loaded liposomes (consisting of lipids (HSPC, cholesterol), DSPE-PEG_{2000}, and DSPE-PEG_{2000}-Maleimide) were found with the help of Design of Experiment. *In vitro* cellular uptake by TNBC cells was confirmed by fluorescent microscopy of liposomes co-encapsulated with Met, and hydrophilic fluorophore. Moreover, the impact of Met-liposomes on TNBC was evaluated by cellular viability assay. To form immunoliposomes, single chain variable fragments (scFvs) were grafted on the liposomes through maleimide-cysteine binding to target trophoblast cell-surface antigen 2 (TROP-2), which is often overexpressed in triple negative breast cancers[4]. Several maleimide:scFv ratios were tested to assess the influence on the physical properties of liposomes, and scFv functionality strength.



$DSPE\text{-}PEG_{2000}$, distearoyl phosphoethanolamine – metoxy poly(ethylene glycol)$_{2000}$; HSPC, hydrogenated soybean phosphatidylcholine; LUV, large unilamellar vesicles; MLV, multilamellar vesicles; PBS, phosphate buffer saline; RT, room temperature; scFv, single chain variable fragment

MET ENCAPSULATED LIPOSOMES

Encapsulation efficiency

$$EE\ (\%) = \frac{m_{Encapsulated\ Met}}{m_{Initial\ Met}} \cdot 100$$

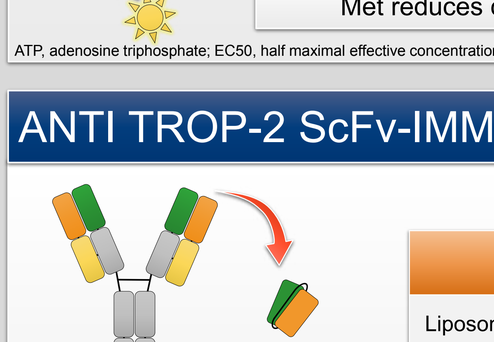
Drug loading

$$DL\ (\frac{mg}{g}) = \frac{m_{Encapsulated\ Met}}{m_{Liposome}}$$

Average physical properties of 3 liposome formulations

D_H (nm)	PdI	ζ (mV)	EE (%)	DL (mg/g)
172.7 ± 12.2	0.096 ± 0.008	-23.3 ± 2.0	2.0 ± 0.2	153.9 ± 11.4

Liposome stability at 4 °C



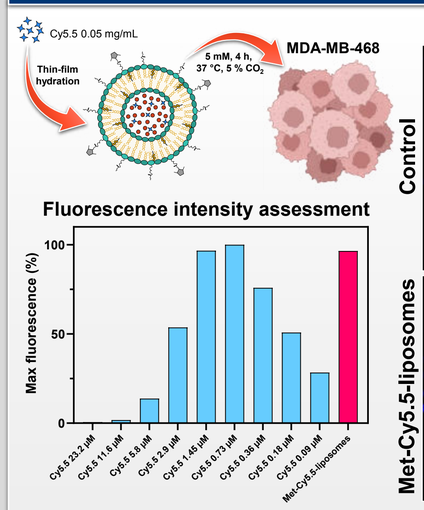
High Met content inside liposomes

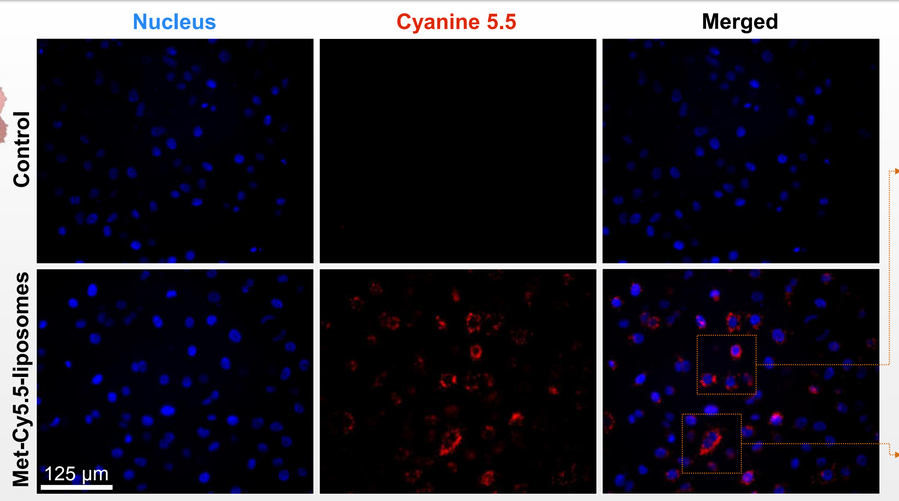
Reproducible formulation

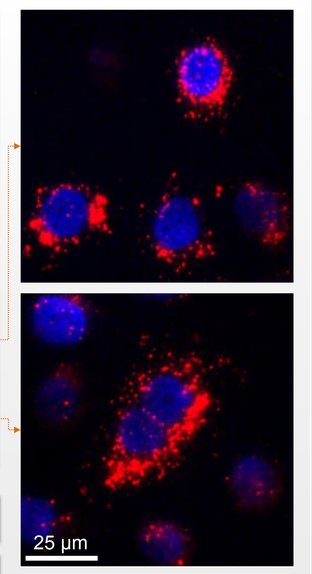
Liposome physical properties are stable over a long period of storage (> 8 months)

D_H, hydrodynamic diameter; DL, drug loading; EE, encapsulation efficiency; Met, metformin HCl; PdI, polydispersity index; ζ, zeta potential

CELLULAR UPTAKE OF MET-LIPOSOMES







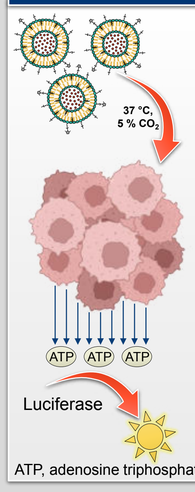
Co-encapsulated Cy5.5 correspond to 0.73 – 1.45 µM (EE 3 – 6 %)

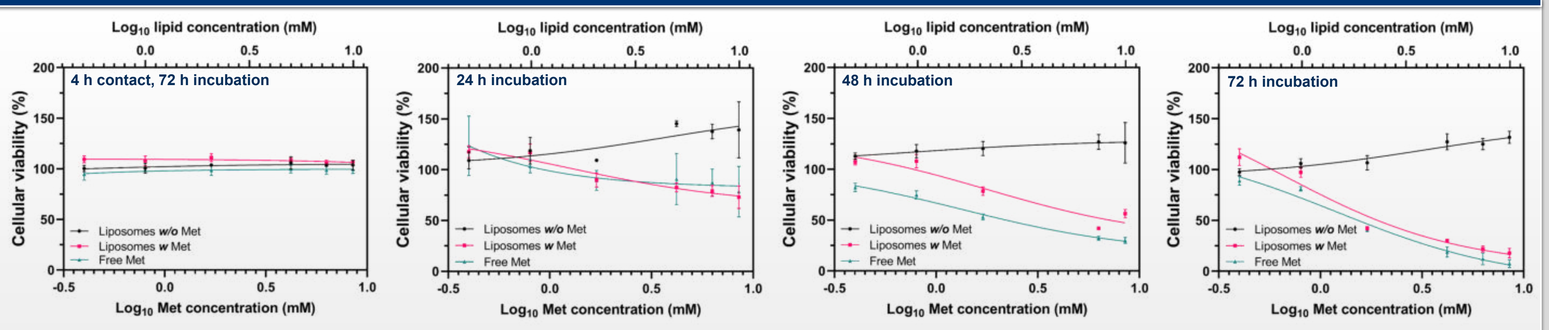
No Cy5.5 fluorescence in cells treated with cell culture media

Met-Cy5.5-liposomes were clustered inside the cytosol around nucleus

Cy5.5, sulfo cyanine 5.5 amine; EE, encapsulation efficiency; Met, metformin HCl

CELLULAR ASSESMENT OF MET-LIPOSOMES





Empty liposomes show no signs of toxicity

$EC50_{Met}$ ~ 1.5 mM for free Met and Met-liposomes

Slope steepness of cellular viability increases with higher incubation time

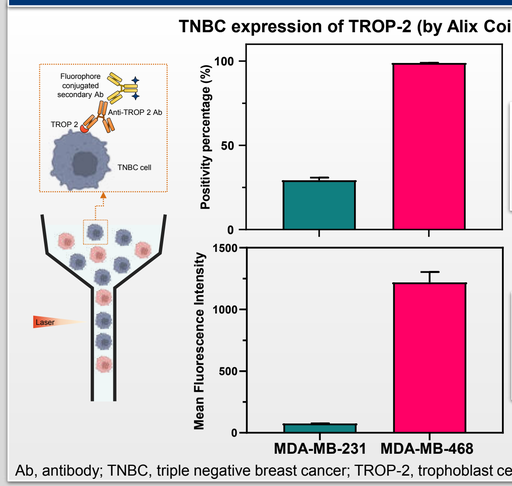
Met reduces cell proliferation

Met-liposomes show similar effect as free Met

ATP, adenosine triphosphate; EC50, half maximal effective concentration; Met, metformin HCl

TROP-2 QUANTIFICATION

TNBC expression of TROP-2 (by Alix Cointet)



All MDA-MB-468 cells strongly express TROP-2 antigen

TROP-2: suitable marker for targeted therapy

Ab, antibody; TNBC, triple negative breast cancer; TROP-2, trophoblast cell-surface antigen-2

ANTI TROP-2 ScFv-IMMUNOLIPOSOMES

PEG_{2000}Maleimide : scFv ratio *mol:mol*

Liposomes *w/o* scFv	Liposomes *w* scFv 1	Liposomes *w* scFv 2	Liposomes *w* scFv 3
0	$750 \cdot 10^3$	$350 \cdot 10^3$	$150 \cdot 10^3$

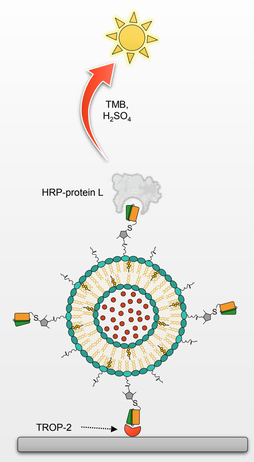
Average physical properties of scFv conjugated liposomes

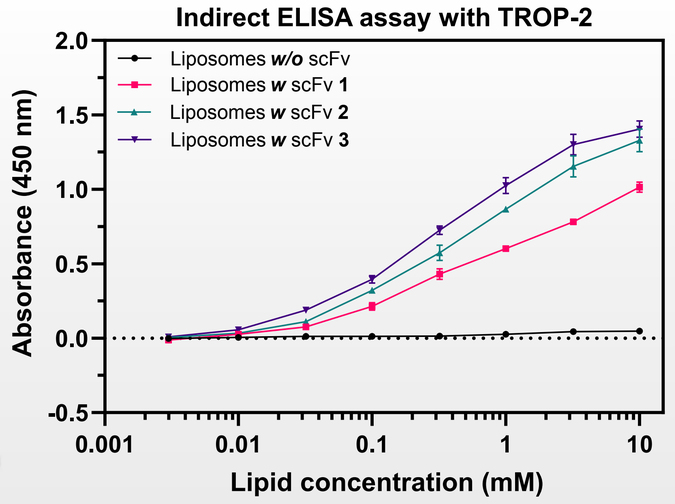
N°.	D_H (nm)	PdI	ζ (mV)	DL (mg/g)
Liposomes ***w/o*** scFv	192.6 ± 1.5	0.073 ± 0.02	-21.0 ± 1.1	125.0 ± 1.3
Liposomes ***w*** scFv 1	192.7 ± 1.3	0.093 ± 0.01	-20.7 ± 1.0	127.4 ± 1.3
Liposomes ***w*** scFv 2	194.3 ± 2.0	0.099 ± 0.02	-19.1 ± 1.3	124.7 ± 0.4
Liposomes ***w*** scFv 3	192.8 ± 2.6	0.096 ± 0.01	-19.8 ± 1.0	133.6 ± 1.2

ScFv conjugation has no impact on physical properties of immunoliposomes

D_H, hydrodynamic diameter; DL, drug loading; PdI, polydispersity index; scFv, single chain variable fragment; ζ, zeta potential

FUNCTIONALITY OF MET-LIPOSOMES-SCFV





Liposomes *w/o* scFv exhibited no absorbance

ScFv functionality is intact after functionalisation with liposomes

Strength of absorbance increases with higher initial scFv quantity

ScFv quantity on liposomes is affected by initial conjugation conditions

HRP, horseradish peroxidase; scFv, single chain variable fragment; TMB, tetramethylbenzidine substrate; TROP-2, trophoblast cell-surface antigen-2

CONCLUSION

In this study we successfully vectorised and optimised Met encapsulation inside highly stable liposomes with a relatively high drug loading. Co-encapsulation of hydrophilic fluorophore allowed to confirm cellular uptake of Met loaded liposomes inside TNBC cells *in vitro*, which lead to reduced cellular viability due to the presence of Met. ScFv conjugation on the surface of the liposomes had no effect on scFv functionality nor the physical properties of Met loaded liposomes. Higher scFv quantity led to an increase in absorbance strength, suggesting higher scFv number on liposomes. Next step in this study is to perform *in vitro* evaluation of scFv conjugated liposomes.

Acknowledgments

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[2] Li, Ying, et al. *Molecular pharmaceutics* 16.7 (2019)
[3] Foretz, Marc, et al. *Cell metabolism* 20.6 (2014)
[4] Aslan, M., *et al.*, *npj Breast Cancer* **7**.141 (2021)