Solid Lipid Nanoparticles for Blood-Brain Barrier Targeted Delivery of Repurposed Drugs in Glioblastoma models

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Introduction and Objective

Glioblastoma (GBM) is a highly aggressive brain tumor with limited therapeutic options, largely due to poor drug penetration across the **blood-brain barrier (BBB)**. **Solid lipid nanoparticles (SLN)** are biocompatible nanocarriers suitable for **lipophilic drug encapsulation**, enabling controlled release and surface functionalization, and can diffuse through biological membranes, including BBB, enhancing **brain drug delivery**(PMID: 34841846). In this study, SLN were developed to deliver **repurposed drugs**(PMID: 34944514) and their efficacy and delivery performance were evaluated in 2D, 3D GBM models, and BBB models.

Repurposed drugs efficacy in GBM cell lines

IC_{50} Values (µM)			
Repurposed Drug	**Solvent**	**U87-MG**	**U251-MG**
Simvastatin (SIM)	Ethanol	0.660	2.858
Activated SIM	H_2O	1.329	12.78
Mebendazole	DMSO	1.05	18.43
Itraconazole	DMSO	> 20	> 20
Clarithromycin	Ethanol	> 20	> 20
Diclofenac	Ethanol	419.5	519
Ketorolac (Toradol®)	H_2O	> 800	NT

Figure 1. IC_{50} values of repurposed Drugs tested in U87-MG, U251-MG cell lines. NT indicates not tested.

SLN Formulation and characterization

Several repurposed drugs were evaluated in GBM cell lines (U87-MG, U251-MG); **simvastatin (SIM)** showed the best efficacy (*Fig 1*).

SLN prepared by hot high-shear homogenization (HHSH) showed spherical morphology (~100 nm), low polydispersity (PDI <0.25) (*Fig 2A & B*), and high Simvastatin encapsulation efficacy (1 mg/mL, EE 99.10%). **Freeze-drying** with cryoprotectants preserved particle size upon reconstitution (*Fig 3*), overcoming instability commonly reported for SLN.

Release was enhanced at acidic tumoral pH vs. physiological pH, indicating selective delivery in the tumor microenvironment (*Fig 4*).

Simvastatin encapsulation into SLN

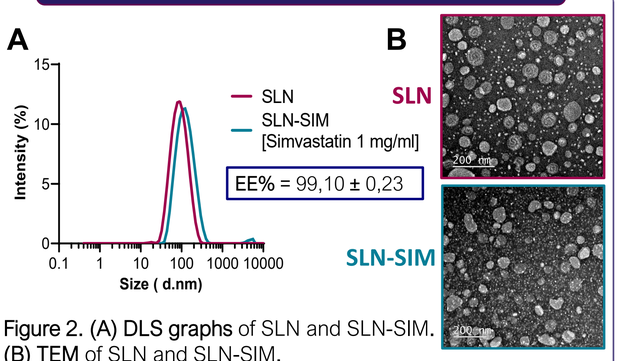

Figure 2. (A) DLS graphs of SLN and SLN-SIM. (B) TEM of SLN and SLN-SIM.

Freeze drying studies

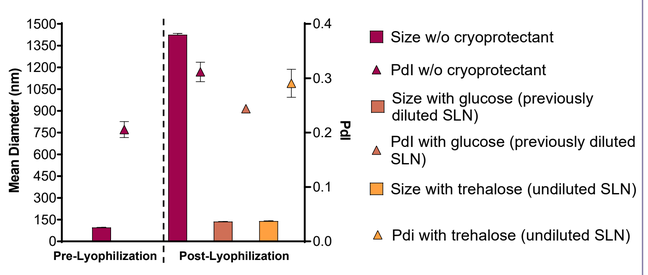

Figure 3. Schematic representation of particle size and polydispersity index (PdI) of SLN before and after lyophilization, with or without cryoprotectants.

Release assay

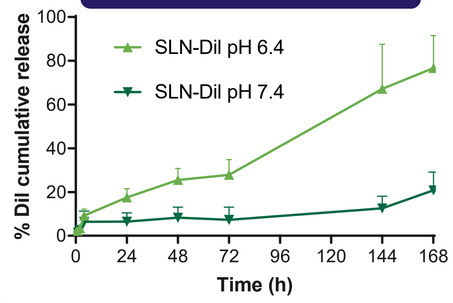

Figure 4. Drug release profile of SLN-DiI nanoparticles over 7 days. Release (%) is shown at pH 7.4 and pH 6.4.Data are presented as mean ± SD (n ≥ 4).

In vitro efficacy

In vitro studies in GBM cell lines showed safe SLN, while SLN-SIM and free SIM showed high therapeutic efficacy (*Fig 5*). These findings were confirmed in scaffold-free 3D GBM spheroid models, where empty SLN induced partial disaggregation and SLN-SIM and free SIM (246.8 µM) showed high therapeutic efficacy (*Fig 6*).

Efficacy in 2D GBM cell lines

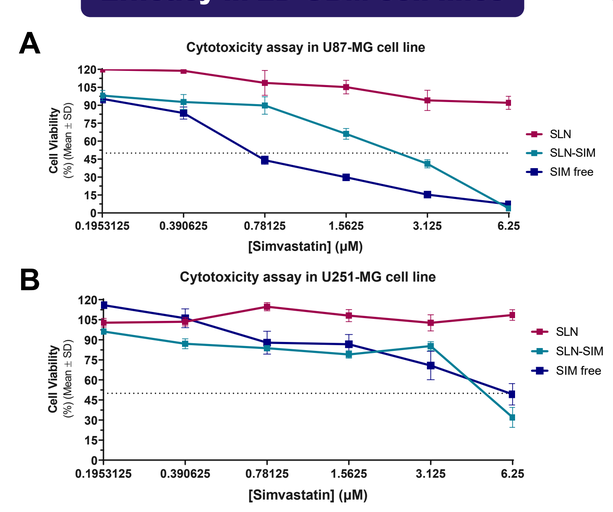

Figure 5. Prestoblue® cytotoxicity assay in U87-MG (A) and U251-MG (B). Cell viability (%) for empty SLN, free SIM, and SLN-SIM. IC_{50} values: U87-MG: 0.85 µM (SIM), 2.28 µM (SLN-SIM); U251-MG: 6.31 µM (SIM), 4.99 µM (SLN-SIM).

Efficacy in 3D GBM spheroid models

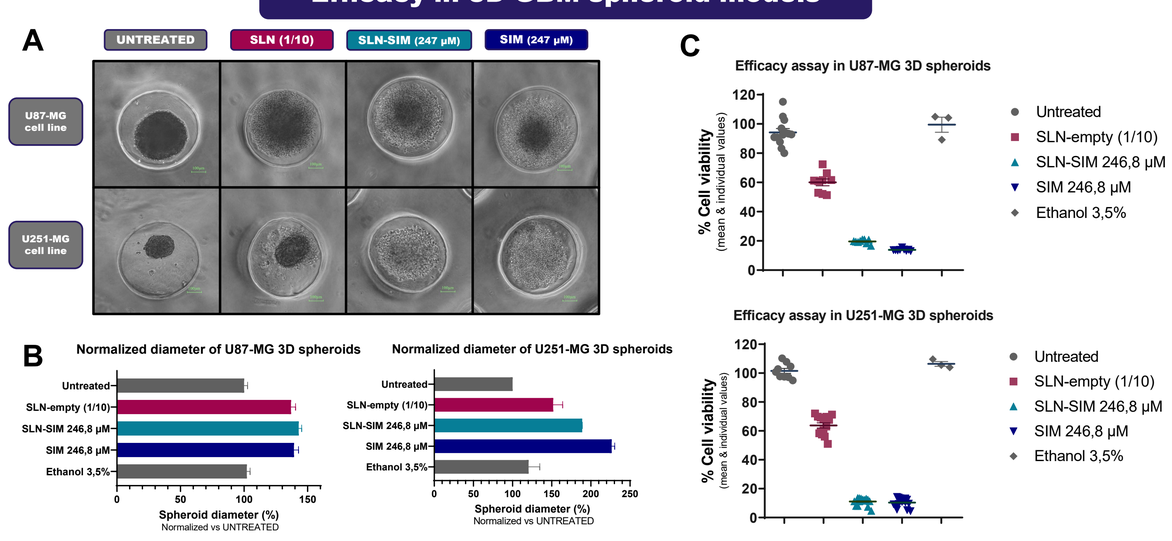

Figure 6. Evaluation of treatment efficacy in scaffold-free 3D GBM spheroid models. (A) Images of U87-MG and U251-MG spheroids after treatment with SLN (1/10), SLN-SIM (246.8 µM), and free SIM (246.8 µM). (B) Quantification of normalized spheroid diameter. (C) Prestoblue® cell viability (%) after treatment. Data presented as mean & individual values (n ≥ 6).

BBB crossing assay

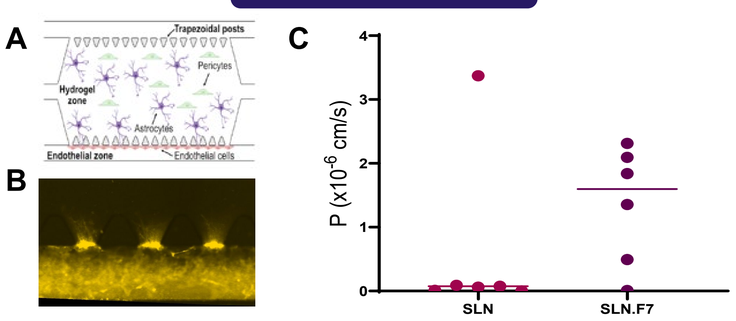

Figure 7. Organ-on-a-Chip BBB model (A&B) for BBB crossing assay (C). Permeability values (P, ×10^{-6} cm/s) as a scatter dot plot with median, n ≥ 6.

Internalization assay

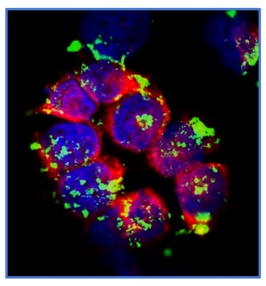

Figure 8. Confocal image of HCT116 cells treated with SLN-F7.

SLN demonstrated transport across a **Organ-on-a-chip BBB model**, with significantly enhanced crossing capacity when modified with the **group-patented cell-penetrating peptide F7** *(Fig 7)*.

F7 conjugation improved SLN cellular uptake in both GBM cell lines (*data not shown*). Internalization was confirmed by **confocal microscopy** *(Fig 8).*

In vivo maximum tolerated dose (MTD)

MTD studies showed **good systemic tolerability**. No significant body-weight changes were observed *(Fig 9)*, and hepatic, renal, and triacylglycerol profiles remained within normal ranges, indicating **absence of systemic toxicity.**

MTD *in vivo* assay

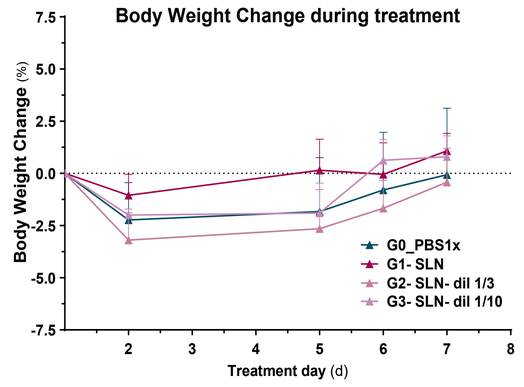

Figure 9. *In vivo* maximum tolerated dose (MTD) study in C57BL/6 mice. Body weight change (%) over 8 days of treatment: G0 (PBS control), G1 (SLN at max dose), G2 (SLN 1/3 dose), and G3 (SLN 1/10 dose). Results as mean ± SEM, n ≥ 4.

Conclusions

- ✓ Functionalized SLN provide a stable, safe, and efficient platform for delivering repurposed drugs in GBM.
- ✓ High encapsulation efficiency, pH-responsive release, and improved BBB transport were achieved.
- ✓ Strong therapeutic efficacy was observed in both 2D and 3D GBM models.
- ✓ These results support functionalized SLN as a versatile, biocompatible platform for **lipophilic drug-delivery** and targeted applications, particularly in **cancer therapy.**