

Supplementary Materials: Liposomes as a Nanoplatfom to Improve the Delivery of Antibiotics into *Staphylococcus Aureus* Biofilms

Table S1. Physicochemical properties of tested antibiotics. daddy.


Antibiotics						
 Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).	Molecular weight (g/mol)	Log P	pKa	Ref.		
	LEV	361.7	2.10	6.24	[47,48]	
	VCM	1485.7	2.03	2.99	[49,50]	
	RFB	847.0	4.10	6.9	[51,52]	

Table S2. Physicochemical properties of LEV-loaded liposomes.

Lipid Composition (molar ratio)	Loading capacity (µg/µmol)	I.E. (%)	Ø (µm) (P.I.)	Zeta Potential (mV)
DMPC:CHOL:SA (7:2:1)	<2	<3	0.13 (<0.1)	+18 ± 1
DMPC:DMPG (8:2)	<2	<3	0.10 (<0.1)	-17 ± 1
DMPC:SA (9.5:0.5)	<2	<3	0.13 (<0.1)	+4 ± 1

Initial lipid concentration, [Lip]_i – 30 µmol/mL; Initial antibiotic concentration [LEV]_i – 1 mg/mL; Loading capacity – (AB/Lip)_f (µg/µmol); I.E. (%) – Incorporation Efficiency, [(LEV/Lip)_f] / [(LEV/Lip)_i] × 100; Ø – mean size; P.I. – polydispersity index; DMPC – dimyristoyl phosphatidyl choline; DMPG – dimyristoyl phosphatidyl glycerol; SA – stearylamine; CHOL – cholesterol; Results are expressed as mean ± SD.

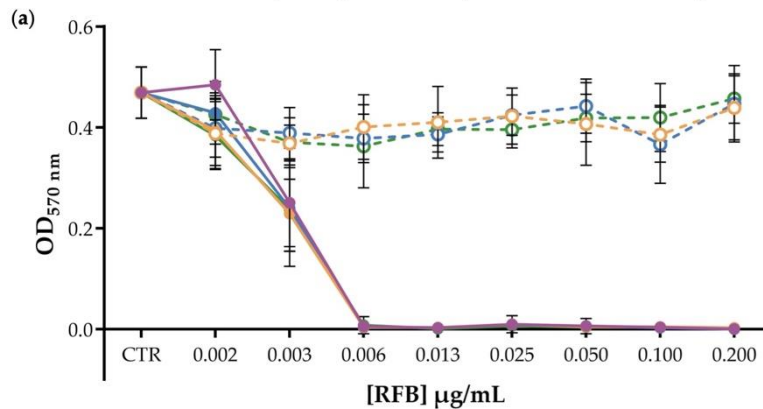
Table S3. Physicochemical properties of rhodamine-labelled liposomes used in influence of lipid composition in *S. aureus* biofilm interaction assays and in biofilm transwell model experiment.

Formulations	Loading capacity (µg/µmol)	RFB (µg) / (0.5 µmol of lipid)	I.E. (%)	Ø (µm) (P.I.)	Zeta Potential (mV)
LIP1 Unloaded	---	---	---	0.12 (<0.1)	-23 ± 1
LIP1 Loaded	8 ± 0.2	4 ± 1	101 ± 8	0.12 (<0.1)	-24 ± 3
LIP2 Unloaded	---	---	---	0.11 (<0.1)	-19 ± 1

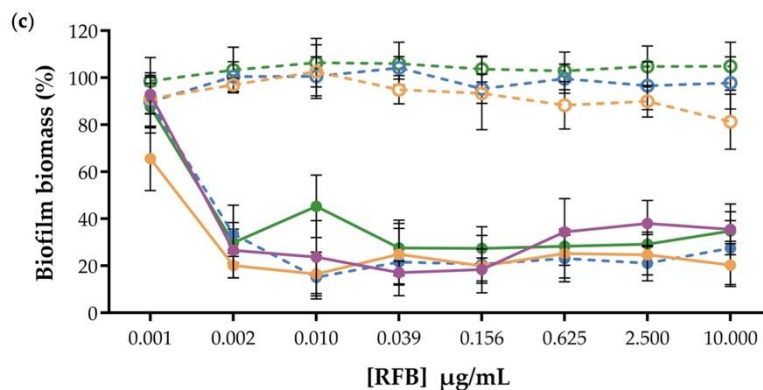
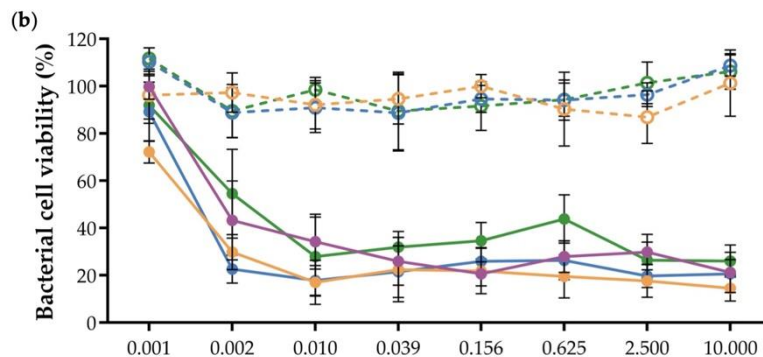
Loaded	13 ± 1.7	7 ± 1	75 ± 1	0.12 (<0.1)	-21 ± 2
Unloaded				0.13 (<0.1)	+12 ± 1
LIP3				0.13 (<0.1)	
Loaded	11 ± 1.3	6 ± 1	57 ± 11	0.13 (<0.1)	+10 ± 1

Initial lipid concentration, [Lip]_i – 30 μmol/mL; Rhodamine concentration: 0.2 mol%; (RFB/Lip)_i – LIP1 = 10 nmol RFB/μmol of lipid, LIP2 = 17 nmol RFB/μmol of lipid and LIP3 = 25 nmol RFB/μmol of lipid; Loading capacity – (RFB/Lip)_f (μg/μmol of lipid); I.E. (%) – Incorporation Efficiency, [(RFB/Lip)_f] / [(RFB/Lip)_i] × 100; Ø – mean size; P.I. – polydispersity index.

Planktonic *S. aureus* susceptibility to free RFB, loaded and unloaded liposomes



***S. aureus* biofilm susceptibility to free RFB, loaded and unloaded liposomes**



- Free RFB
- LIP1
- RFB LIP1
- LIP2
- RFB LIP2
- LIP3
- RFB LIP3

Figure S1. Susceptibility of planktonic and biofilm *S. aureus* to RFB-loaded liposomes. (a) *In vitro* planktonic MSSA susceptibility assessment through broth microdilution method followed by turbidity measurement ($OD_{570\text{ nm}}$), after 24 h of incubation with free RFB and loaded and unloaded formulations (0.002 – 0.200 $\mu\text{g/mL}$). The positive control corresponds to untreated planktonic MSSA in MHB represented by CTR. (b) Determination of viable bacterial cells (MTT assay) and (c) biofilm biomass quantification (CV method) after performing the broth microdilution method with a RFB concentration range of 0.001 to 10.000 $\mu\text{g/mL}$, against mature MSSA biofilm. Results are expressed as mean \pm SD of at least three independent experiments.

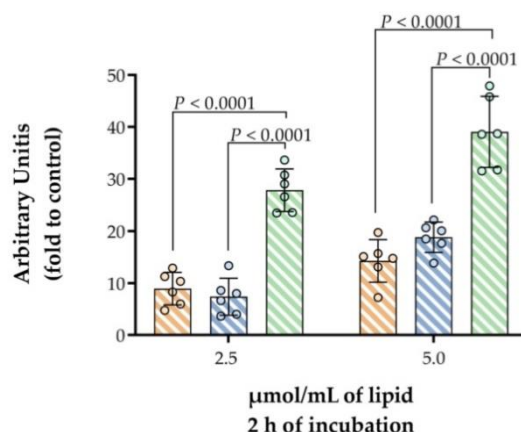


Figure S2. Influence of lipid composition on *S. aureus* biofilm interaction. Mature MSSA biofilm incubated with the LIP1, LIP2 and LIP3 at 2.5 and 5.0 $\mu\text{mol/mL}$ of lipid for 2 h. Results are expressed as mean \pm SD of at least three independent experiments. Statistical comparisons were determined by two-way ANOVA (Tukey's multiple comparisons test) analysis of variance compared between formulation groups.

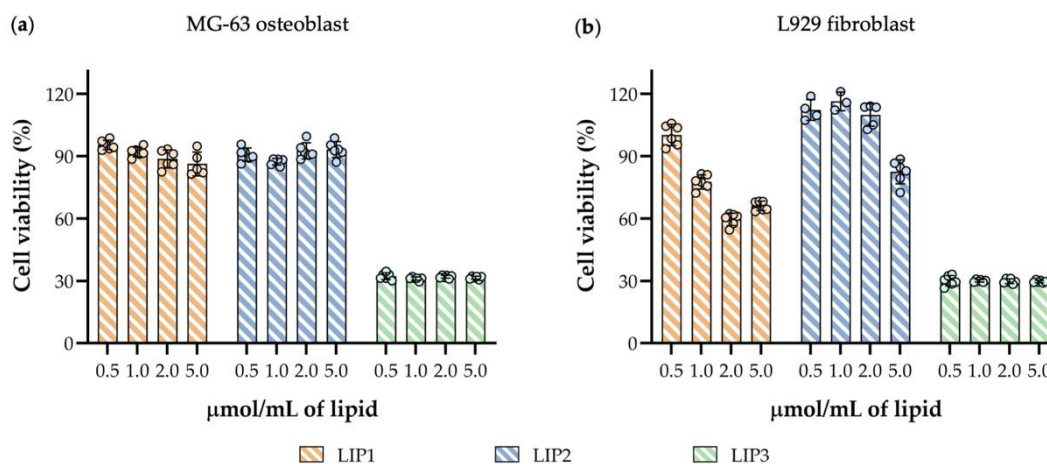


Figure S3. Cell viability of (a) human MG-63 osteoblast cell line and (b) mouse L929 fibroblast cell line, 24 h after incubation with unloaded liposomes, LIP1, LIP2 and LIP3 at lipid concentrations of 0.5, 1.0, 2.0 and 5.0 $\mu\text{mol/mL}$. Cell viability was determined by MTT reduction assay. Results are expressed as mean \pm SD of at least two independent experiments.