Comparative Evaluation of Liposome-Encapsulated Photosensitizers for Photothermal & Photodynamic Therapy Against *Staphylococcus aureus* Biofilms

Paria Mojarrad[1], Deborah A. Nkansah[1], Timo Laaksonen[1,2], Päivi Tammela[1], Zahra Gounani[1]

[1] Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, 00790 Helsinki, Finland

[2] Faculty of Engineering and Natural Sciences, Tampere University, 33720 Tampere, Finland

BACKGROUND

700K AMR deaths per year (2014)

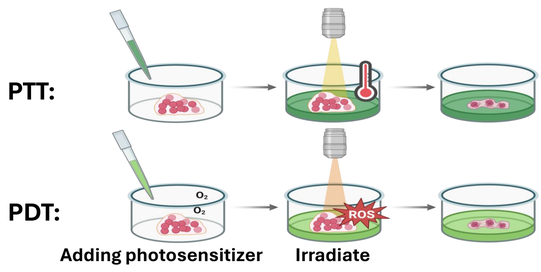
10M/year Predicted deaths by 2050

80% Of infections involve biofilms

- Antimicrobial resistance (AMR) is a global health crisis, predicted to surpass cancer mortality by 2050
- *S. aureus's* resistant strains are among **WHO high-priority pathogen**.
- *S. aureus* biofilms are 1000× more resistant than planktonic cells to treatments.
- Biofilms protect bacteria from antibiotics and the immune system, causing **chronic, hard-to-treat infections**

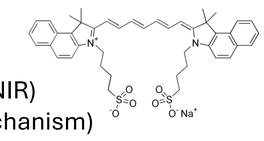
PTT & PDT; SMARTER APPROACHES

- Works against **drug-resistant strains**
- **Rapid** bactericidal effect
- **Low chance of resistance development**
- Inactivates virulence factors
- Partially controlled with **light-activation**



PHOTOSENSITIZERS (PSS):

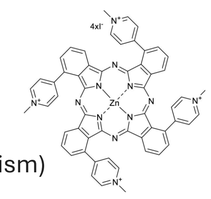
ICG - Indocyanine Green



FDA-approved dye

- Peak absorption at **~800 nm** (NIR)
- Converts light → **heat** (PTT mechanism)

LASU - Zinc Phthalocyanine



Novel tetracationic photosensitizer

- Peak absorption at **~670–690 nm**
- Converts light → **ROS** (PDT mechanism)

METHOD

1) **Liposome preparation:** Thin film formation→ Hydration→ Extrusion
2) ***S. aureus* biofilm formation**
3) **Irradiation**
4) Measuring biofilm susceptibility endpoint parameters, minimum biofilm inhibitory light dose (**MBiLD**), and minimum biofilm eradication light dose (**MBeLD**)
5) **Other measurements:** colony counting, temperature measurement, viability assay

LIPOSOME PROPERTIES

Positive zeta potential → enhanced interaction with negatively charged bacterial membranes

IRRADIATION PARAMETERS

PS	Conc.	Irradiation parameters		
		λ (nm)	Time (min)	Power (mW/cm²)
ICG	0.1 mg/mL	808	1, 3, 10	500, 750, 1000, 1250, 1500, 2000
LASU	0.2 & 0.4 mg/mL	689	3, 10, 20, 30	100, 400, 1000

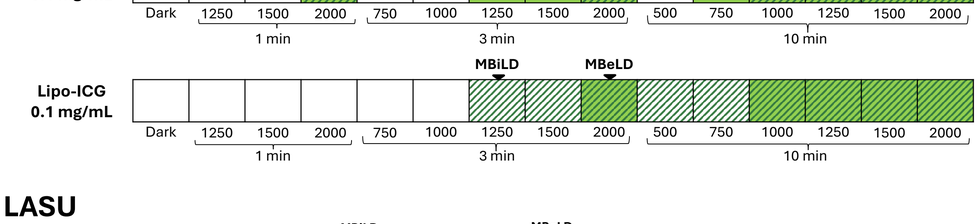
- **IEC 60825-1 MPE:** 200 mW/cm² for skin at both 689 nm & 808 nm. Powers above MPE are *in vitro* only.

MPE: Maximum Permissible Exposure

Research Question: Do light-activated photosensitizers, free and liposome-encapsulated, effectively combat *S. aureus* biofilms?

RESULTS

PSs Absorbance Curves



Liposome Properties

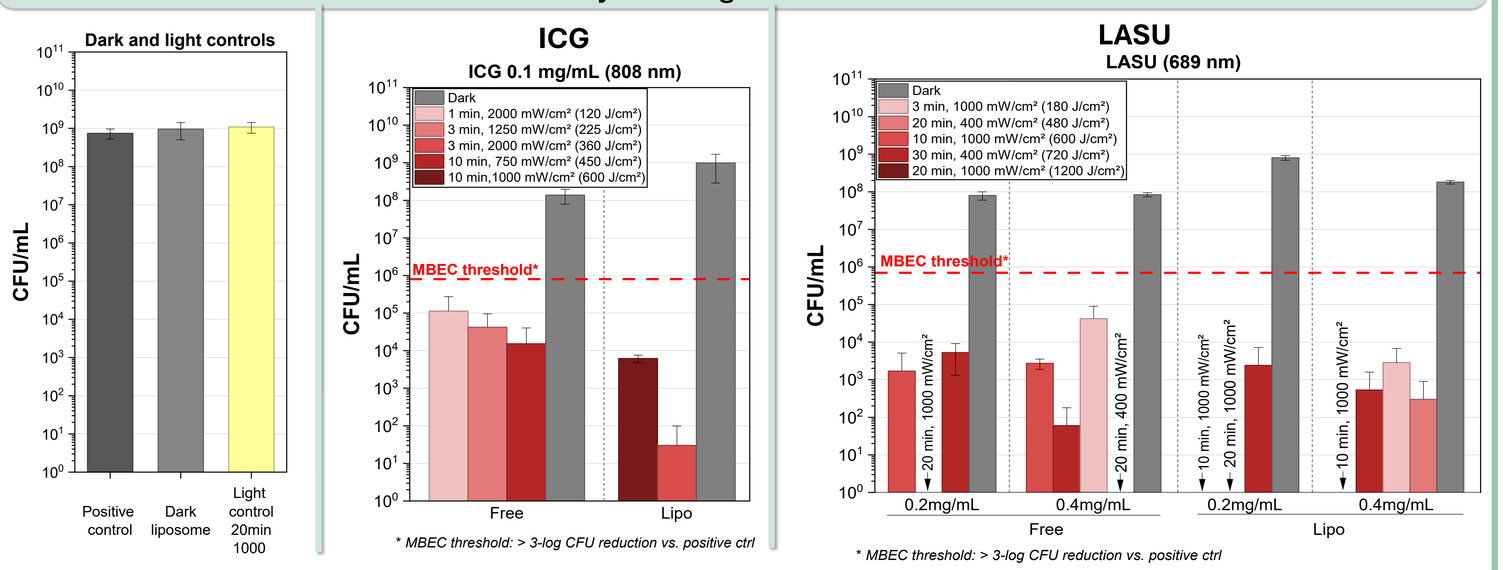
Liposome formulation	Properties	
	Size (nm)	Zeta (mV)
Lip-ICG 0.1 mg/mL	154.1 ± 64.9	+15.0 ± 0.6
Lip-LASU 0.2 mg/mL	116.9 ± 13.6	+18.1 ± 1.4
Lip-LASU 0.4 mg/mL	143.8 ± 25.7	+19.0 ± 0.7

- All liposomal formulations exhibited nanoscale particle sizes (117–154 nm) and positive zeta potentials (+15 to +19 mV), indicating successful formulation

Biofilm Inhibition and Eradication (MBiLD, and MBeLD)

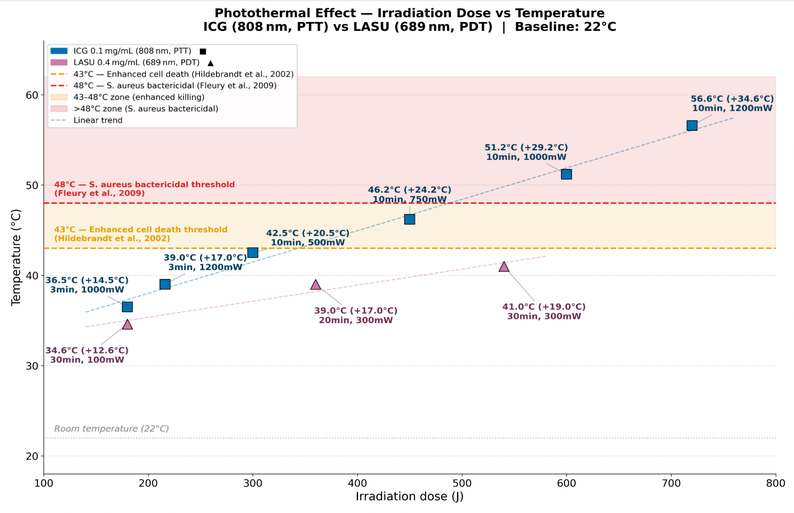
- Liposomal ICG showed superior inhibition, while free ICG was more effective for eradication. As ICG is anionic and the liposome cationic, the formulation is near-neutral. **The more anionic free ICG penetrates the biofilm EPS more deeply**, improving eradication.
- Free LASU was more effective for inhibition, with no added benefit from encapsulation in eradication. **The more cationic Lipo-LASU likely adsorbs to the biofilm surface** without penetrating deeper, so free and liposomal LASU eradicate comparably.

Colony Counting of MBeLD Results



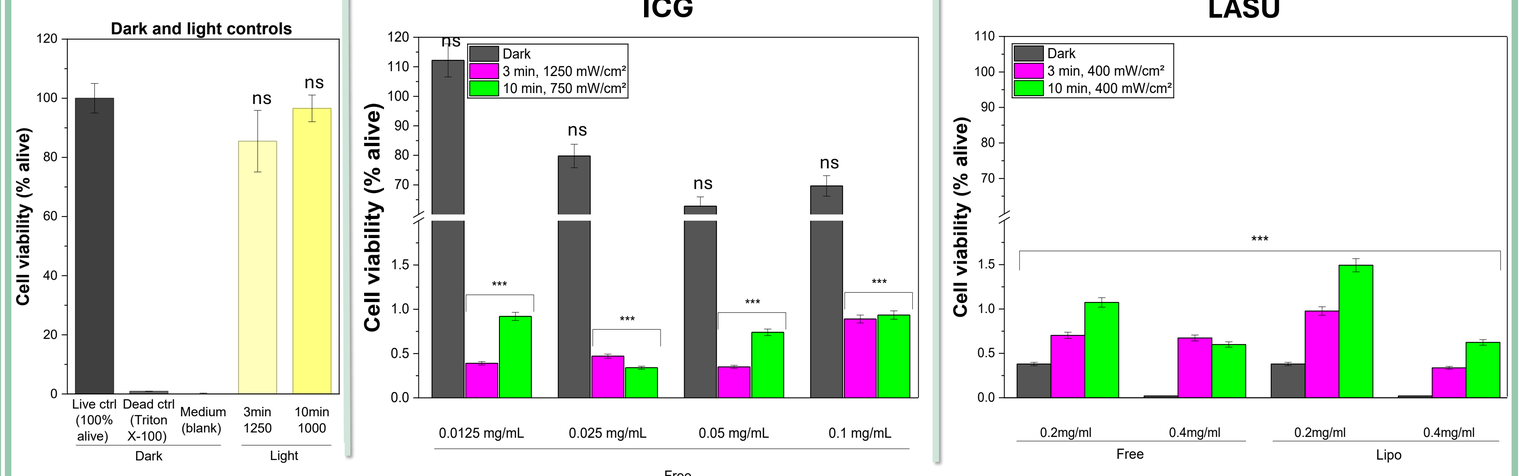
- The cell count for all controls (untreated biofilm, liposome, and light control) reached to approximately 10^9 CFU/mL.
- CFU/mL counts confirmed MBEC validity, with all conditions showing less than 3-log reduction relative to the positive control.

Photothermal Effect of ICG



- ICG exceeded the 48°C *S. aureus* bactericidal threshold (51.2°C at 1000 mW/cm²; 56.6°C at 1200 mW/cm², 10 min).
- LASU reached 41°C even after 30 min long irradiation at 300 mW/cm², confirming it effects via photodynamic rather than photothermal mechanisms.

Cell Viability Using AlamarBlue



- Light irradiation alone had no cytotoxic effect on HDF cells, confirming safety of the light
- LASU demonstrated intrinsic dark toxicity, whereas ICG was non-toxic in the absence of irradiation.
- Both LASU and ICG induced complete cytotoxicity in HDF cells upon light irradiation.

CONCLUSION

- ❖ Both photosensitizers demonstrated strong light-dependent killing.
- ❖ For LASU, the free form showed greater inhibitory potency, while eradication efficacy was comparable between free and liposomal formulations.
- ❖ The surface charge of molecules might play a role in their anti-biofilm efficiency.
- ❖ LASU's sensitivity to even minimal light irradiation makes it suitable for medical device sanitization, wound infection treatment, and self-disinfecting hospital surfaces.

REFERENCES

1. Shirzad M, et al. ICG-mediated PDT reduces MRSA drug resistance. *Photochem Photobiol.* 2021.
2. Leitão MM, et al. LASU quarantine dye: antimicrobial phthalocyanine activated by indoor light. *ACS Appl. Mater. Interfaces.* 2021.
3. Leitão MM, et al. LASU for self-disinfecting anti-COVID textiles. *Dyes Pigm.* 2022.
4. IEC 60825-1. Safety of laser products – Equipment classification. 2014.

Paria.mojarrad@helsinki.fi

HELSINGIN YLIOPISTO
HELSINGFORS UNIVERSITET
UNIVERSITY OF HELSINKI
FARMASIAN TIEDEKUNTA
FARMACEUTISKA FAKULTETEN
FACULTY OF PHARMACY