Supplementary Information for "Rational Control of Combined Photothermal and Photodynamic Therapy for Effective Eradication of Biofilms"

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Figure S1. Binding exotherms of ALA to PAA-SPIONs detected via isothermal titration calorimetry (ITC).

Strong binding exotherms observed throughout the titration confirmed successful binding of ALA to PAA-SPIONs as shown in Supplementary Figure S1.



Figure S2. Dose-dependent cytotoxicity results determined by MTT assay on L929 cells.

The dose-dependent cytotoxicity of free ALA, PAA-SPIONs and ALA/PAA-SPIONs was first evaluated on a healthy cell line (L929) at different concentrations (21-336 µg ALA /mL or 37.5-600 µg Fe /mL using standard MTT assay after 24 h incubation. No significant toxicity was detected in the studied concentration range as shown in Supplementary **Figure S2**, confirming that free ALA, PAA-SPIONs and ALA/PAA-SPIONs do not induce toxicity in the dark.



Figure S3. Photothermal screening during laser treatment

Temperature increases during laser irradiation of *P.aeruginosa* biofilms that were treated with free ALA, PAA-SPION, and ALA/PAA-SPION ([Fe]: 600 µg/ml, [ALA]: 2 mM ALA) were monitored with a thermal camera. Cells without laser treatment were used as control and the temperature increase of laser treated cells with respect to the control as shown in Supplementary Figure S3.

Modelling Temperature Dynamics in Photothermal Therapy

For simulating the light-to-heat conversion of nanoparticles, we created 2 simulation setups in ANSYS Lumerical Software. First, we used ANSYS Lumerical FDTD Solver and parametrized the periodic unit cell volume of given simulation to mimic different concentrations of solution. Thickness is defined with single side length of unit cells, each contains one particle inside, as thin film. If the concentration of the solution is very high and the volume fraction of nanoparticles are comparable to medium volume, FDTD solution might be not sufficient due to scattering and plasmonic effects in a periodic structure. To validate FDTD simulation, we setup a second simulation using Effective Medium Theory and ANSYS Lumerical Stack Solver for creating a homogenous effective medium.

In our Periodic FDTD simulations, we defined a cubic unit cell for each particle. the relationship between the number of particles, volume and concentration was determined based on the following formula:

For 1 Particle
$$\rightarrow$$
 V = $\frac{\Box}{\Box \Box}$

Concentration (ug/mL)	Volume for 1 particle
200	(348.55 nm) ³
600	(241.67 nm) ³
1000	(203.83 nm) ³

Table 1 Simulation Volumes for different concentrations

where *C* represents the concentration, *W* is the molecular mass (5100 kDa = 5100000 g/mol), *V* is the volume of the simulation region, and *A* is Avogadro's number. Consequently, the concentrations used and their corresponding minimum simulation region volumes for a single particle are (348.55 nm)³ for 200 μ g/mL concentration, (241.67 nm)³ for 600 μ g/mL concentration, and (203.83 nm)³ for 1000 μ g/mL concentration as shown in the Table 1. The nanoparticles are modeled as 3D spheres with a diameter of 12.4 nm. These particles are dispersed in water, serving as the medium. The simulation region was set to a 3D space of 100x100x100 nm³ boundary conditions were periodic in the x-y plane and perfectly matched layers (PML) in the z-direction. A planewave source, covering wavelengths from 450 to 1000 nm, was used for excitation. For further investigation of concentration's effect on absorption efficiency, 640 nm excitation wavelength is chosen. The mesh was set to an automatic non-uniform configuration with a maximum granularity of 0.5 nm in the nanoparticles.

For Stack Solver simulations , the effective medium was computed using the Maxwell Garnett Approximation defined for spherical inclusions [1]. The effective permittivity of new medium was calculated using the following equation:

$$\Box_{\Box\Box\Box} = \Box \left(\frac{\epsilon_2 + 2 \epsilon_2 + 2f (\epsilon_2 - \epsilon_1)}{\epsilon_2 + 2 \epsilon_1 - f (\epsilon_2 - \epsilon_1)} \right)$$

Here, ϵ_1 and ϵ_2 represent the permittivity's of the host medium (Water) and the spherical inclusions (nanoparticles), respectively, while *f* denotes the volume fraction of the inclusions. Absorption ratio of effective medium is calculated using ANSYS Lumerical Stack Solver, and Effective medium is represented as a 2D thin film placed in host material. Thickness of 2D material is chosen to be same as thickness of FDTD simulation for consistency. Overall, 2 Simulation model is summarized in figure S4.



Figure S4 Models used to simulate light absorption of nanoparticles with different concentrations. a) cubic unit cell of FDTD model with periodic boundaries in x-y plane, PML boundary in z plane. Simulation Region contains one particle at the center and concentration is controlled by changing the volume of the unit cell. b) Effective Medium Model for 2D analytical solution of absorption with different concentrations. Similar to FDTD model, concentration is controlled using volume fraction "f" of nanoparticle compared to the host medium in a cubic unit cell. Effective dielectric constant of the composite material, host medium, nanoparticle defined as " ϵ_{eff} ", " ϵ_2 ", and " ϵ_1 ". Both Model Simulated with same thickness "a" in z-direction , which is one side length of defined unit cubic cell and excited with a plane wave source in z-direction.

Beer Lambert's Law for Fixed Thickness

Our both FDTD and Effective Medium Model have concentration depended on thickness. For fixed thickness of solution, we simply applied Lambert's Law, which shows linear relationship between the concentration of particles and absorption. However, this approach might not fully capture the complexities of heat dispersion, as shown by T. G. Mayerhöfer at al (2019) [2]. In contrast, our initial simulations with adjusted thicknesses align more closely with experimental observations, encapsulating an interaction between nanoparticle concentration and heat dispersion. To demonstrate both scenarios for comparison, we applied linearity correction of absorption calculation by Beer's Law [3] for our Effective Medium Simulations with fixed thickness as:



Normalized Absorption and Light-to-Heat Conversion

 $dQ_{injected} = dQ_{dissipated} + m_{PAA-SPION}C_{PAA-SPION}dT + m_{water}C_{water}dT$

Here, the injected heat from laser is proportional to the laser power density (I, W/m²), duration (t) and absorption (A). Thus, the energy fluence $(\Box, \Box/\Box 2)$ of laser excitation at a given wavelength is given by

 $(J/m^2) = A \times I \times t$. The injected heat is then found by multiplying the energy fluence with the illuminated volume V. Since the thermal simulations are done in a volumetric bath, laser energy is given in terms of $\frac{\Box}{\Box \Box^3}$ for given unit volume of photonic simulation using the Beer's Law:

 $dQ_{injected} = E_{density} \times$ Illuminated Surface Area × thickness

We assumed that the laser is uniformly absorbed by the medium, although it might differ in vivo experiments.

We quantified the light-to-heat conversion of nanoparticles in our FDTD and Effective Medium solution by first calculating the Energy density ($E_{density}$) of given excitation wavelength using absorption (A), Laser Power (P) and excitation duration (t).

$$E_{density} = A \times P \times t$$

Total injected heat is then found by multiplying Energy density Illuminated Volume. Since the thermal simulations are done in a volumetric bath, Power is given in terms of $\frac{W}{cm^3}$ for given unit volume of photonic simulation using the Beer's Law.

$$Q_{injected} = Power \times time$$

 $Q_{injected} = E_{density} \times Illuminated Total Surface Area \times D$

Finally, heat change is found by $Q = m C \Delta T$ for given solution mass (m) and specific heat (C) with dissipation term:

 $Q_{\text{injected}} = Q_{\text{dissipated}} + m_{\text{PAA}-\text{SPION}}C_{\text{PAA}-\text{SPION}}\Delta T + m_{\text{water}}C_{\text{water}}\Delta T$

 $Q_{dissipated} = Q_{Raditaion} + Q_{Convection}$

since $m_{water} \gg m_{PAA-SPION}$

 $\Delta T = \frac{dQ_{injected} - dQ_{dissipated}}{m_{PAA-SPION} C_{PAA-SPION} + m_{water} C_{water}} \approx \frac{Q_{injected} - Q_{dissipated}}{m_{water} C_{water}}$

Thermal Simulation with Boundary Conditions for Dissipation

We have already calculated the $\Box_{injected}$ using photonic simulation, which is total heat transferred to the volume. For calculating $\Box_{\Box\Box\Box\Box\Box}$ and heat transfer, we used Lumerical Heat Solver Software. Overall simulation regions and boundary conditions is summarized in Figure S5. For Heating and Cooling of 5 minutes, 10 minutes, 15 minutes, 20 minutes is simulated and temperature at the center is plotted for analysis.



Figure S5 Simulation Region and Boundary Conditions for proposed Thermal Simulation. Cylindrical Region is the illuminated region with Laser Power Input.

General Heat Equation

In lumerical, heat transport solver calculates the solution T (the temperature) to the heat transport equation in a solid medium as:

$$\rho c_{p} \frac{\Box T}{\partial t} - \nabla \cdot (k \nabla T) = Q$$

where ρ is the mass density (kg/m³), cp is the specific heat (J/kg.K), k is the thermal conductivity (W/m.K), and Q is the applied heat energy transfer rate (W/m³).

Boundary Conditions

1. Fixed Temperature B.C. (Dirichlet Boundary Condition)

This boundary condition is used for fixing the temperature in surrounding ambient (or reservoir) as :

$$T(x) = T_{\text{fixed}} \text{ for } x \in \partial \Omega$$

2. Insulator Boundary Condition (Neumann Boundary Condition)

An insulator boundary condition is applied when there is no heat flux across the boundary, effectively modeling an insulated surface. This is represented by setting the normal derivative of the temperature field to zero:

$$-k \frac{\Box T(x)}{\partial n} = 0 \text{ for } x \in \partial \Omega$$

Where,

k : thermal conductivity

 $\frac{\Box T(x)}{\Box n}$: derivative of the temperature in the direction normal to the boundary

3. Convective Boundary Condition (Robin Boundary Condition)

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Convective Boundary Condition models the heat transfer between the surface and a fluid moving over it. The heat flux is proportional to the difference between the surface temperature and the fluid temperature. It's given by:

$$-k \frac{\Box T(x)}{\partial n} = h(T(x) - T_{\infty}) \text{ for } x \in \partial \Omega$$

Where,

 \boldsymbol{h} : convective heat transfer coefficient

 T_{∞} : the far-field (or fluid) temperature

4. Radiation Boundary Condition

thermal radiation exchange between a surface and its surroundings or between two surfaces. The Stefan-Boltzmann law is used as:

$$-k \frac{\Box T(x)}{\partial n} = \epsilon \sigma \Big(T(x)^4 - T^4_{surrounding} \Big) \text{ for } x \in \partial \Omega$$

Where,

 $\boldsymbol{\varepsilon}$: emissivity of the surface

 σ : Stefan-Boltzmann constant

References for Heat Equations:

- 1. Wolfram Research, Inc., "Heat Transfer," in Wolfram Language & System Documentation Center. [Online]. Available: <u>https://reference.wolfram.com/language/PDEModels/tutorial/HeatTransfer/HeatTransfer.html#</u> <u>531721061</u>.
- 2. Ansys, Inc., "Boundary Conditions in HEAT Simulation Object," Ansys Optics, 2024. [Online]. Available: <u>https://optics.ansys.com/hc/en-us/articles/360034398314-Boundary-Conditions-in-HEAT-Simulation-Object</u>.